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MECHANISM OF THE FORMATION OF PURE CHOLESTEROL GALLSTONES

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Stones formed in the gallbladder without the coexistence of inflammation resulting from infection are usually designated as "pure" stones, for example, "pure cholesterol stones" or "pure calcium pigment stones." The cholesterol stones, which are by far the most common example of this type, consist largely of an enmeshing network of radial crystals of cholesterol, together with small amounts of alkali and calcium cholates and bile pigments. Since the pure cholesterol stones always occur singly, Meckel von Hemsbach [1] called them cholesterol solitaires.

In a recent study of certain colloidal phenomena which occur in the formation of gallstones accompanied by infection,[2] it was disclosed that there was no satisfactory mechanism to explain the formation of pure cholesterol stones. In the present article an attempt is made to give the experimental basis of a colloidal theory of the process of formation of such concretions.

Although the colloidal theory of the formation of gallstones is largely the product of recent investigations, it should be recalled that what is now termed colloidal behavior was recognized as important in producing concretions by Hippocrates, the Father of Medicine, in the fourth century B. C., and by Galen in the second century of our era. These renowned physicians of ancient Greece attributed the abnormal deposits to an accumulation of mucus which clung to the organ and served as a nucleus for the stone which subsequently formed. The first experimental evidence of the rôle which colloids may play in the formation of the concrements was obtained in 1684 by von Heyde, who dissolved out the crystalline material from a urinary calculus and observed a residual framework. This organic framework was recog-

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1. von Hemsbach, Meckel: Mikrogeologie, 1856.
2. Weiser, H. B., and Gray, G. R.: J. Phys. Chem. **36**:286, 1932.

nized clearly by Meckel von Hemsbach,[1] as evidenced by the following quotation:

> Two basic factors underlie the formation of every true gall or urinary stone; first, the presence of an organic substance, mucus, in which there may be deposition of salts; second, a suitable urinary or gall fluid to serve as the mother liquor for these sediments. The decomposable organic substance, mucus, is unquestionably necessary, because urinary salts and gall substances of themselves can yield only crystalline, pulverulent or granular precipitates and never larger pieces. Stones are formed only when an organic binder is carried down too.

Although the presence of a colloidal organic binding material resulting from an inflammatory process has been definitely established as essential for the formation of certain types of concretions, it was demonstrated almost a quarter of a century ago by Aschoff and Bacmeister [3] and by Schade [4] that both gall and urinary calculi may form under suitable conditions without inflammation as a result of infection.

The incidence of pure cholesterol stones is believed by Aschoff [5] to result from a disturbed metabolism which gives an abnormally high content of cholesterol in the blood and subsequently in the bile. According to Dostal and Andrews,[6] a survey of the experimental evidence does not support this view; on the contrary, it shows the absence of any connection between the cholesterol content of blood and bile. Recently, however, Wilkie and Doubilet [7] at McGill University made striking observations which support Aschoff's theory. Thus it was shown that in normal animals with the cystic duct tied, cholesterol passes from the blood through the mucosa of the gallbladder into the bile provided the cholesterol concentration of the bile is lower than that of the blood, whereas cholesterol passes from the bile through the gallbladder into the blood stream provided the cholesterol concentration of the bile is higher than that of the blood. Moreover, the amount as well as the direction of passage of the cholesterol appears to depend on the blood-bile cholesterol ratio.

In contrast to Aschoff's theory, Naunyn [8] attributed the presence of excess cholesterol which may lead to the formation of stones in the gallbladder to the disintegration of the epithelium of the gallbladder or to the direct secretion of cholesterol by the mucosa of the gallbladder.

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3. Aschoff, L., and Bacmeister, A.: Die Cholelithiasis, Jena, G. Fischer, 1910. Kleinschmidt, O.: Die Harnsteine, Berlin, Julius Springer, 1911.

4. Schade, H.: (*a*) Kolloid-Ztschr. **4**:175, and 261, 1909. (*b*) Alexander, J.: Colloid Chemistry New York, The Chemical Catalog Company, 1928, vol. 2, p. 803.

5. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 206.

6. Dostal, L. E., and Andrews, Edmund: Arch. Surg. **26**:258, 1933.

7. Wilkie, A. L., and Doubilet, Henry: Arch. Surg. **26**:110, 1933.

8. Naunyn, B.: Klinik der Cholelithiasis, Leipzig, F. C. W. Vogel, 1892.

This view has been largely disproved, and has been abandoned by most pathologists. Recently, however, it has been revived by Elman and Graham [9] to account for the presence of cholesterol crystals in the walls of the gallbladder. Although this pathologic condition called cholesterosis or "strawberry" gallbladder is well known, it does not follow that the crystals deposited in the mucosa of the gallbladder are secreted by the mucosa.

On one point everybody is agreed: Whatever the source of the excess cholesterol, a condition must arise which causes precipitation of the compound or no stone can form. During stasis, Aschoff visualizes the precipitation from a hypercholesterolated bile about some nucleus, ultimately leading to the formation of a gallstone. While this step is necessary, pathologists have not always recognized that precipitation is in itself altogether inadequate to account for the binding of the minute crystalline particles into a concretion. Schade [4b] realized the necessity of accounting for the collection of the precipitated cholesterol into a coherent mass and proposed the following mechanism:

> Bile always contains, in addition to cholesterol, small quantities of fat dissolved in its cholate. Now in stasis of the bile, as the experience of the surgeon and the pathologist proves, the concentration of the cholate is gradually diminished by autolysis and resorption until finally a water-clear, almost cholate-free fluid is left; but the cholesterol remains in undiminished quantity and is ultimately in excess. The increasing impoverishment of the bile in cholate content, compels small quantities of cholesterol to separate out from time to time. But owing to the presence of fat it is guttulate separation which occurs, and since in such simple stasis, foreign substances are lacking, there is nothing to prevent the aggregation of the droplets.

While Schade's proposed mechanism possesses elements of value, it is based on certain misconceptions which render it inadequate. In the first place, it is taken for granted that the alkali cholates can hold large amounts of cholesterol in the dispersed state; but it requires about 40 parts of cholate to disperse 1 part of cholesterol. In the next place, it is assumed that during stasis the cholates gradually disappear, allowing the cholesterol to precipitate. But Rous and McMaster [10] and more recently, Andrews, Dostal, Goff and Hrdina [11] demonstrated conclusively that stasis alone does not necessarily alter the bile salt-cholesterol ratio in the gallbladder. The introduction of some other factor such as bacterial infection or chemical irritation, say by pancreatic juice, is necessary for resorption of the bile salts to take place. But inflammation from infection, or, indeed, anything more than a relatively mild chemical inflammation, is not essential for the formation of pure cho-

9. Elman, Robert, and Graham, E. A.: Arch. Surg. **24**:14, 1932.
10. Rous, Peyton, and McMaster, P. D.: J. Exper. Med. **34**:47, 1921.
11. Andrews, Edmund; Dostal, L. E.; Goff, M., and Hrdina, L.: Ann. Surg. **96**:615, 1932.

lesterol solitaires, since gallbladders in which they are found usually show no signs of inflammation and the pathologic results of infection are absent. Moreover, in the presence of infection, stones of a distinctly different type are formed. Finally, emphasis is laid on the importance of the presence of excess fat; but cholesterol is quite soluble in fat, and an excess of the latter would tend to prevent rather than favor the formation of gallstones.

EXPERIMENTAL WORK

The following experiments appear to throw considerable light on the mechanism by which precipitated cholesterol may be collected into a unified coherent mass:

1. It is commonly stated that cholesterol, which is quite insoluble in water, is held in the dispersed state in the bile by the peptizing action of the alkali cholates. This statement we have found to be only partly correct. Fat-free cholesterol was digested for several days in a 6 per cent solution of sodium glycocholate kept at 37 C., the body temperature. The amount taken up corresponds to but 0.2 Gm. in 100 cc. of solution or 1 part of cholesterol to 30 parts of bile salt. In contrast to the relatively low peptizing action of the cholates is the marked solubility of cholesterol in fats. Thus, at body temperature, 100 cc. of olive oil dissolves 6 Gm. of cholesterol. Since normal bile may contain 1 per cent or more of fatty material, it is apparent that from one-third to one-half the cholesterol in normal bile from the common duct [12] is dissolved in the emulsified fat. Moreover, any excess cholesterol tends to concentrate around the fat droplets.

It is significant that the cholesterol which separates from a supersaturated solution in fat comes out in the form of needle-like interlacing crystals and not in the form of thin platelets such as those obtained from the alcoholic solution.

2. A drop of olive oil was shaken with 10 cc. of a 6 per cent solution of sodium glycocholate. A stable emulsion was formed in which the droplets varied in size from extremely minute to fairly coarse. Figure 1 *A* shows the appearance of a portion of this emulsion magnified 70 times. The emulsion is quite stable but tends to "cream" on standing, giving an upper layer that is relatively richer in fat than the lower layer.

3. A drop or two of olive oil saturated with cholesterol at 50 C. was added to 10 cc. of the solution of sodium glycocholate at 25 C. and the mixture shaken vigorously. An emulsion was again formed, but this time there was present an amount of free cholesterol in excess of the amount required to saturate the fat at 25 C. A small portion of this excess cholesterol was peptized by the alkali cholate and the remainder concentrated on the surface of the droplets of emulsified fat. This is shown clearly in figure 1 *B*. It is interesting to note that under these conditions the fat droplets have an "armor plate" of cholesterol, as evidenced by the fuzzy appearance of the edges of most of the fat droplets and by the distortion in shape of many of them. It is apparent that cholesterol has a marked tendency to collect at an oil-water interface. For this reason, in the bile excess cholesterol over and above that dissolved in the bile fat and peptized by the alkali cholates concentrates at the surface of the droplets. It thus appears that the chief rôle of the bile cholates in holding relatively large amounts of cholesterol in colloidal

12. The cholesterol content of normal bile from the common duct is between 0.06 and 0.12 per cent.

dispersion is as an emulsifying agent for the fats, thereby furnishing a relatively large surface on which minute particles of cholesterol are absorbed and prevented from settling out.

4. Like most emulsions, those considered in the preceding paragraph "cream" on standing; that is, the upper portion becomes relatively richer in fat. This process is accompanied by some coalescence, especially of the larger drops, into still larger units. When the droplets are coated with a film of cholesterol there is a marked tendency to form clumps of droplets. Thus, after an emulsion containing excess cholesterol has stood for a day or two, it is apparent that a considerable portion of the cholesterol has collected in an upper cloudy layer. Examination under the microscope reveals the presence of clumps of droplets

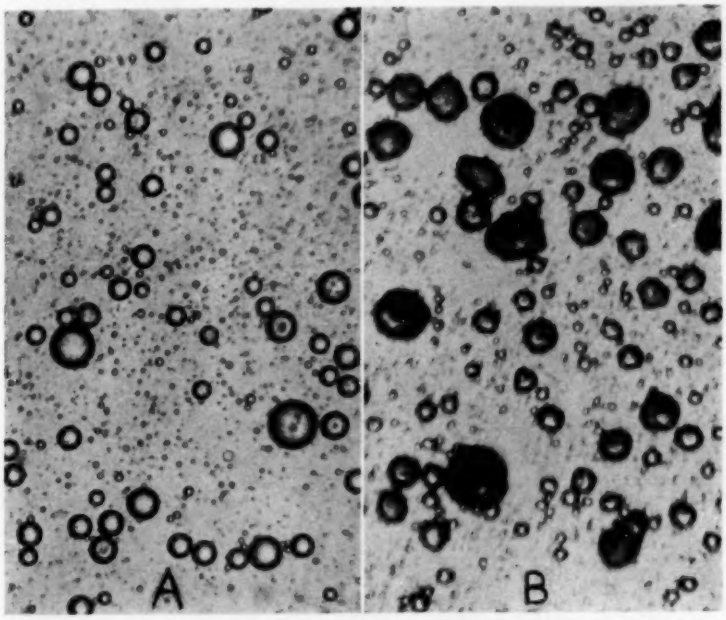

Fig. 1.—Emulsions of olive oil with sodium glycocholate: *A*, in the absence of cholesterol; *B*, in the presence of excess cholesterol; × 70.

coated with cholesterol so finely divided that no crystal structure is visible. A typical clump of this kind is shown in figure 2 *A*. Note the irregular shape of the clump and the white color which is due to the minute particles of cholesterol in the oil-water interface.

5. For the formation of large cholesterol crystals from the minute particles, it is necessary for the emulsifying film of sodium glycocholate to be broken so that cholesterol can come in contact with the saturated fat droplets. Under such circumstances it would be expected that the minute particles would dissolve and reprecipitate out on larger units. There proved to be two relatively simple methods of breaking the glycocholate film on the droplets: First, add a trace of acid which converts the glycocholates to glycocholic acid which is not an emulsifying agent; or second, allow the sample to dry, thus causing the film of the hydrophilic emulsifying agent to crack.

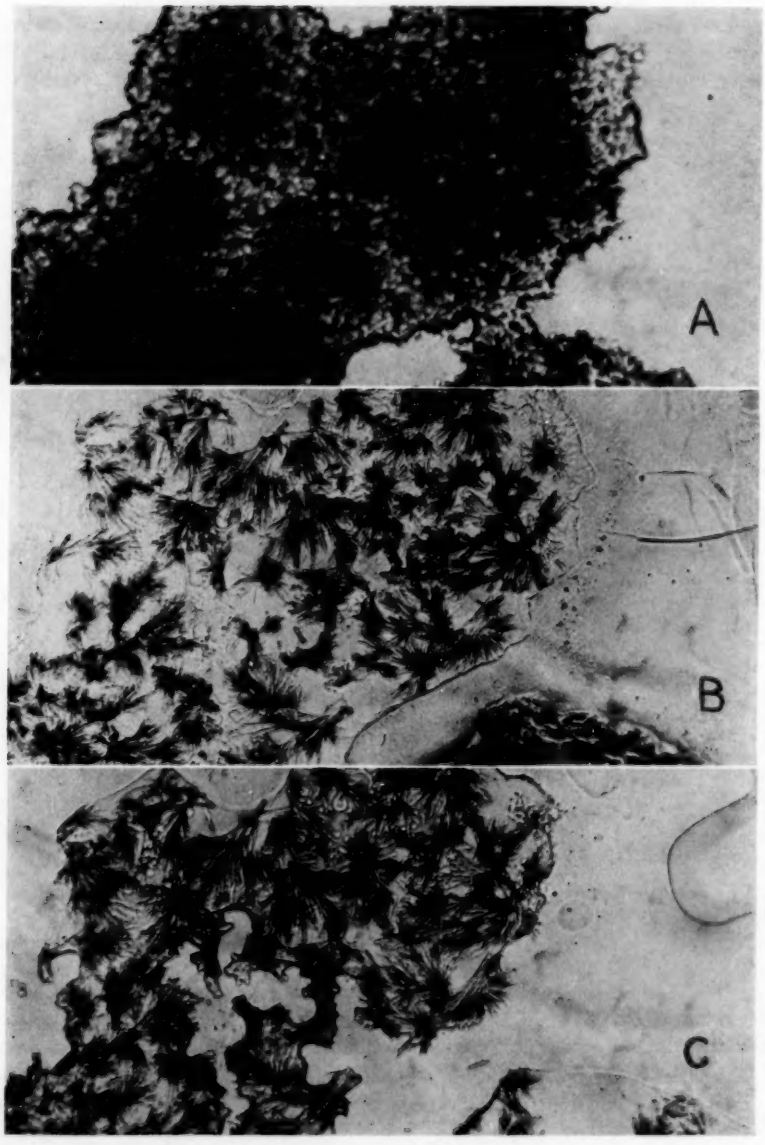

Fig. 2.—Cholesterol-fat clump: *A*, freshly formed; *B*, after four hours; *C*, after twenty hours; × 70.

The effect of allowing the specimen shown in figure 2 *A* to stand in the air is shown strikingly in figure 2 *B* (after four hours) and 2 *C* (after twenty hours). Note the disappearance of the minute particles of cholesterol and the formation in their stead of the fanlike, feathery crystals similar in all essential respects to those which characteristically separate from the solution in fat. This process is accompanied by the release of the greater portion of the fat solvent. Note the relatively large size of the crystals, especially in *C*, and that they are suspended in a drop of fat the outline of which is clearly visible.

6. It would appear obvious that the formation of a mass of interlocking crystals by the mechanism described in the previous paragraph would yield a relatively firm "stone." This was demonstrated by pouring fat supersaturated with cholesterol into glycocholate solution, emulsifying, allowing to stand for some time, and then separating the mass of fat and cholesterol from the remainder of the emulsion by centrifugating. After absorbing the excess fat with blotting paper, it was formed into a ball and dried. The result was a relatively hard crystalline mass, simulating the natural pure cholesterol stone in appearance, composition and properties. To prepare a photograph, a small ball of the cholesterol mass was flattened out on a microscope slide, dried, and digested at body temperature for four weeks. The result is shown in figure 3. Although the magnification is only 10 diameters, the radial crystalline structure characteristic of the natural stones is clearly revealed. Figure 4 is a section of the preparation shown in figure 3, magnified 70 times. The higher magnification shows in a striking way how a coherent body is obtained as a result of the laying down of an interlacing mass of needle-like crystals.

In the light of the foregoing series of experiments, the mechanism of the formation of cholesterol stones in the absence of an inflammation due to infection is believed to be as follows:

In biliary stasis resulting from anatomic or physiologic abnormalities,[13] the bile collects and concentrates in the gallbladder where it may remain for a long period. During this period of stasis there may be an infiltration of cholesterol from hypercholesterolated blood and a decrease in the amount of the alkali cholates which are responsible for retaining the fat in the form of an emulsion as well as the cholesterol in the dispersed state. In the absence of infection, a decrease in the alkali cholates may result from either or both of the following causes: (1) a change in the *p*H of the bile from the alkaline to the acid side thereby converting the alkali salt to the insoluble glycocholic acid which is neither an emulsifying agent for fat nor a peptizing agent for cholesterol; or (2) a physiologic change in the wall of the gallbladder which allows resorption of the alkali cholates.

There is no doubt that the first factor mentioned will contribute to the precipitation of cholesterol, since bile from the gallbladder is normally acid while that from the hepatic duct is alkaline. This normal change in *p*H value is probably accentuated in biliary stasis.

In the normal gallbladder there is little or no resorption of alkali cholates. Since pure cholesterol stones are found in gallbladders that show no signs of inflammation, past or present, it follows that any resorption of cholates that leads to the formation of a pure cholesterol stone must result from a physiologic derangement that does not produce histologic changes. Clinical irritation that is not sufficiently severe to leave a permanent change in the tissue may be a contributing factor. Thus a reflux of pancreatic juice into the gallbladder causes

13. Aschoff,[5] p. 194.

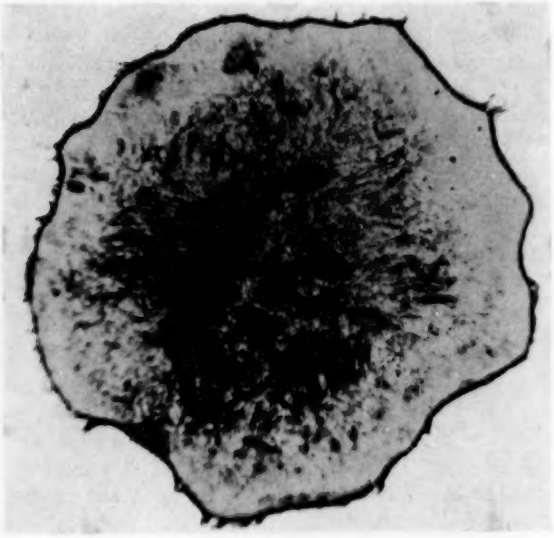

Fig. 3.—Synthetic pure cholesterol stone; × 10.

Fig. 4.—Interlacing crystalline structure of synthetic cholesterol stone; × 70.

cholecystitis and results in marked absorption of cholates.[14] The injection of bacteria produces a similar effect, but this changes the whole picture, causing lesions and leading to a different type of stone.

The disappearance of alkali cholate, either by conversion to glycocholic acid or by resorption, causes precipitation of cholesterol. This is, of course, most marked in highly hypercholesterolated bile. The excess cholesterol collects around the fat droplets which tend to coalesce as the cholate is gradually removed. Clumps of fat interspersed with cholesterol result, and the process of solution of the finely divided particles and subsequent reprecipitation in large needle-like crystals binds the mass together. The continuation of this process for a long period leads eventually to the concrement which consists of relatively large crystals of cholesterol together with a small amount of enclosed fat.

Particular attention should be called to the importance of fat in the synthesis of pure cholesterol stones. Not only does it serve as a collecting agent which brings together the particles of precipitated cholesterol but its solvent action is responsible for the growth of the interlacing crystals which bind the mass into a concrement.

SUMMARY

The results of this investigation may be summarized as follows:

1. Precipitation of cholesterol in the gallbladder is in itself altogether inadequate to account for the formation of pure cholesterol concretions.

2. Experimental observations have been made which furnish the basis of a mechanism to account for the formation of such concretions during biliary stasis resulting from anatomic or physiologic abnormalities.

3. By the proposed mechanism, gallstones have been synthesized which simulate the natural concretions in both macroscopic and microscopic appearance and in properties.

4. Particular attention has been called to the importance of fat in the formation of pure cholesterol concretions, both as a collecting agent for the minute particles of precipitated cholesterol and as a solvent which is responsible for the growth of interlacing crystals into a concrement.

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14. Wolfer, J. A.: Surg., Gynec. & Obst. **53**:443, 1931. Andrews, Dostal, Goff and Hrdina.[12]

HISTOLOGIC OBSERVATIONS IN A CASE OF OLD GUNSHOT WOUND OF THE BRAIN

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The opportunity to study the pathologic aspects of an ancient traumatic lesion of the brain is not frequently afforded one, even though an abundance of neuropathologic material is available for study. It is perhaps for this reason that so little is known about the ultimate appearance and structure of this type of lesion. Aside from a revelation of the behavior of the individual elements after so long an interval, a more comprehensive conception of the life history of cerebral wounds is to be gained from a critical histologic study of such cases when they do come to hand.

The case here considered is of interest from the standpoint of its twenty-two years' duration, the minimal damage to the skull and dura and the comparatively uncomplicated character of the lesion produced by a bullet of small caliber. It was anticipated that an entirely quiescent lesion would be found histologically after this long interval. The indications of long-continued cellular activity and observations which suggested the fate of the various elements made it seem worth while to report the case.

REPORT OF CASE

A Mexican laborer, aged 57, died of lobar pneumonia six days after admission to the Los Angeles County General Hospital. Twenty-two years before he had been accidentally shot in the head while hunting and had remained unconscious for three weeks thereafter. Examination revealed a depression in the skull in the upper left parietal region close to the midline. There were characteristic manifestations of a lesion of the left upper motor neuron—spasticity of the extremities on the right side, atrophy from disuse, hyperactive deep reflexes in these members and pathologic responses of the toes.

Autopsy was performed seven hours post mortem by one of us (T. S. K.). The original defect in the skull had been closed by the formation of new bone, apparently derived from the inner table. There still remained a depression in the outer table. The dura was adherent to the bone at this point. There were no adhesions between the dura and the brain. In the upper part of the left precentral gyrus, at the point of entrance of the bullet, there was found an angular hollowed defect in the cortex

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measuring 2.2 by 1.8 cm. in surface extent and about 0.5 cm. in depth. The pia-arachnoid appeared to be continuous with a thin yellowish membrane which lined the defect. A somewhat distorted 22 caliber bullet was found lodged in a conical cavity in the third right temporal convolution just in front of the preoccipital notch. This defect measured 0.7 by 1 cm. in its surface diameters.

The brain was sectioned along the course of the bullet to expose as much as possible of its track (fig. 1). The bullet, having entered the upper portion of the left precentral gyrus, had emerged from the cortex of the posterior portion of the left cingulate gyrus, penetrated the corpus callosum at the point of union of this structure with the crurae of the fornix, passed through the right lateral ventricle at the juncture of the body, posterior and inferior horns, and penetrated the substance of the right temporal lobe at this surface, where it was found on external examination.

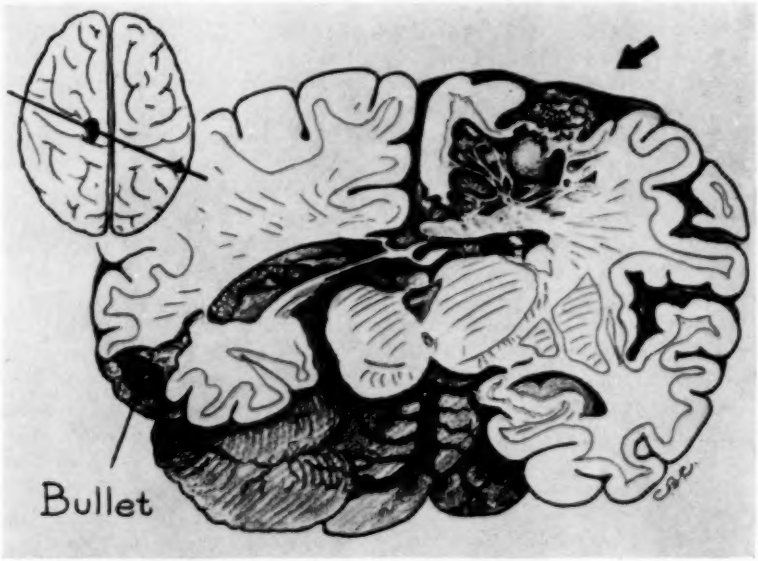

Fig. 1.—Section of the brain along the course of the bullet. The depression in the cortex at the missile's point of entrance, the subjacent multilocular cyst, the depression in the atrophied corpus callosum, the adherent choroid plexus and the cavity of final lodgment of the bullet are indicated. The insert shows the line of section.

At the point of entrance of the bullet, surrounding the depression were found multiple small cystic spaces occupying almost the entire centrum above the corpus callosum. These small spaces had evidently been filled with fluid in the recent state. The intervening walls and lining of the spaces were golden yellow. This extensive lesion was probably the result of indriven fragments and hemorrhage, in addition to the wound made by the bullet. The corpus callosum was atrophied, and the point of entrance of the bullet was marked in this situation by a depression in its upper surface. The choroid plexus was still adherent at the point of exit of the bullet from the ventricle. The cavity in the left temporal lobe in which the bullet was found was conical, with its apex at the ventricle wall and the base

at the surface of the brain. The base of the left cerebral peduncle, the left side of the pons and the pyramid were smaller than the right, owing to secondary degeneration of the motor fibers.

HISTOLOGIC OBSERVATIONS

Blocks of tissues were taken from the cystic area below the wound of entrance, from the cortex along the dorsomesial margin of the hemisphere above the defect, from the corpus callosum at the point of its penetration by the bullet, from the point of adhesion of the choroid plexus to the ventricular wall and from the walls of the cavity in which the missile had finally lodged. Tissue sections from these blocks were stained or impregnated with hematoxylin and eosin, scarlet red, phosphotungstic acid hematoxylin, the cyanine method for tigroid substance, the Courville-Krajian method for myelin sheaths, Krajian's silver impregnation method for reticulin, Cajal's gold chloride method for neuroglia, the reduced silver method for axis cylinders and Penfield's combined method for oligodendroglia and microglia.

Alterations in the Nerve Cells.—In all of the sections studied, the nerve cells, regardless of position, showed a general loss of tigroid substance. In view of the fact that the patient died of lobar pneumonia, this is only of incidental significance. The reduced silver method showed an intact fibrillar apparatus in the nerve cells at a distance, but in those adjacent to the injury, this structure was found to be undergoing regressive change or was absent altogether. In a portion of cortex cut off by the track of the bullet, the nerve cells were morphologically intact, but their fibrillar apparatus was found to be granular. In a limited portion of cortex which formed the dorsal border of the hemisphere, separated from the centrum by the multilocular cavity, the nerve cells were entirely absent.

In a small residual fragment of cortex subjacent to the wound of entrance and separated from the underlying white substance by extensive destructive change, the normal structures were entirely replaced by a loose vascular connective tissue reticulum. In the superficial portion of the opposed cortex, there was a zone of vascular connective tissue which replaced all other elements. The nerve cells in the deeper layers appeared distorted and sclerotic.

In the cortex adjoining the cavity containing the bullet, cyanine preparations revealed the nerve cells to be only faintly outlined, tigroid granules being entirely absent from their cytoplasm (fig. 2 *A*). Some of the cells contained localized collections of pigment. The reduced silver preparations revealed a variable degree of granular disintegration of the neurofibrillar apparatus. Occasional pyknotic or "sclerosed" cells with their characteristic corkscrew apical processes were observed near the margin of the defect. Rarely a deeply impregnated, distorted form was found, the scarred remains of a ganglion cell (fig. 2 *B*).

Alterations in the Nerve Fibers.—The nerve fibers separated from their nerve cells had long since disappeared, so that it was impossible to trace the course of degenerated bundles such as the motor pathway. The white substance somewhat removed from the immediate zone of injury appeared homogeneous, and individual fibers revealed no evidence of injury. In the myelin sheath preparations, as the margin of the bullet track was approached, the nerve fibers became fewer and more altered. Individual fibers were found to be altered by the formation of globular or fusiform swellings of various sizes, characteristic of fibers undergoing regressive change (fig. 2 *C*). At times these swellings occupied the termination of the demonstrable sheath. Rarely an additional unstained portion could be discerned under reduced light, extending toward the margin of the defect. The

terminal portion was frequently found to be paler than usual, at times fragmented or segmented, and often of smaller caliber than the rest of the fiber. This terminal segment was frequently broken off sharply, with a resulting squared end, or presented an irregular, pale, expanded portion varying in size (fig. 3).

The changes in the axis cylinders were equally interesting. In the zone of degeneration these structures were found to be undergoing granular, fragmentary or "burnt string" degeneration. In expanded regions, which corresponded with the myelin swellings, were found local dispersions of the argentophilic material in the form of rings, irregular aggregations or complex masses. The impression given by these collections was that in the process of regressive change the myelin swellings had distorted the arrangement of the rows of argentophilic granules

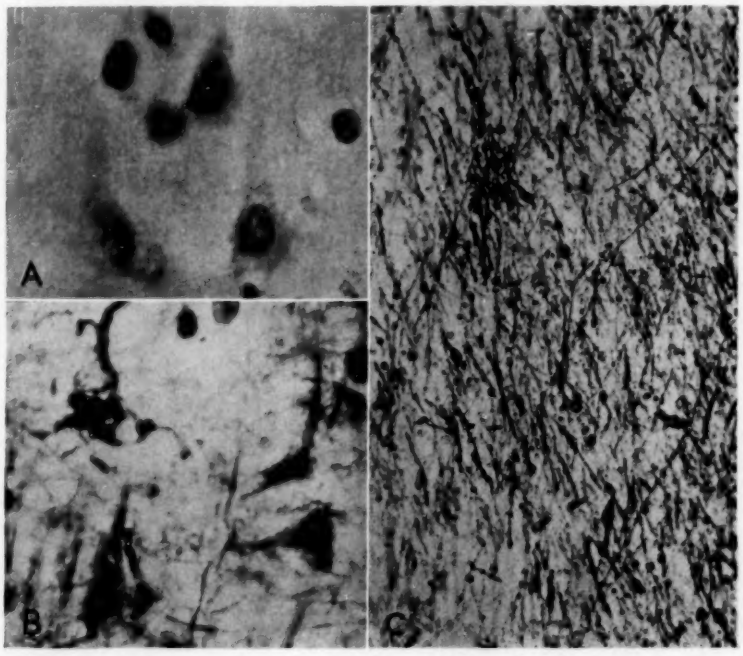

Fig. 2.—Changes in the nerve elements. *A* shows ghost cells at the margin of the cavity in which the bullet was lodged. Cyanine stain for tigroid substance; × 360. *B* shows degenerated nerve cells from the same location. A sclerosed and vacuolated cell is shown in the upper left corner. Cajal's reduced silver method; × 360. *C* illustrates degenerating myelin sheaths from the same situation. Courville-Krajian method; × 120.

representing the remains of the axis cylinder. When occurring at the end of a degenerating fiber, these structures gave rise to appearances of "pseudo end-bulbs."

End-bulbs which are known to persist for months or even years after the injury of the brain were not observed except in a very modified form. At the termination of the fine axis-cylinders was found a small irregular expanded portion, a spear head, a fine hairlike filament or a clump of granules. At times a row of rounded fragments gave the fiber a barred or beaded appearance. The accom-

panying drawing illustrates a number of these terminations of axis-cylinders (fig. 4). In a portion of cortex isolated from the white centrum by local cysts, the axis-cylinders of the morphologically preserved nerve cells were found to terminate a short distance below the cell in a sharp point, a late "corrosion point."

Progress of Phagocytosis.—Substantiating these indications of a long-continued degeneration in the nerve fibers, evidences of active phagocytosis of the products of regressive changes were to be seen. Specific stains revealed globules of free fat in wandering macrophages in the zone of degeneration, especially in the perivascular spaces. The endothelial cells of the blood vessels likewise contained fat.

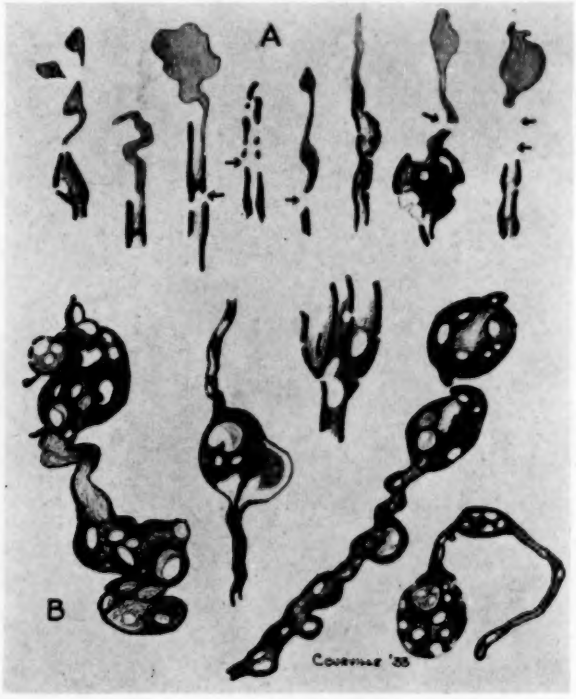

Fig. 3.—Alterations in the myelin sheaths. *A* shows the modes of termination of the fibers extending into the margin of the defect. The arrows indicate fractures in the sheath, possibly artefacts. *B* shows the detail of the globular swellings of the myelin sheaths. Drawn from a Courville-Krajian myelin sheath preparation.

The reduced silver method revealed granules of argentophilic material in free macrophages and, often in larger amounts, in the vascular endothelium.

Of particular interest in this connection was the reaction of the microglia in the cortex adjacent to the track of the bullet. These cells were found to be undergoing morphologic alteration recognized to be transitional in the formation of compound granular corpuscles. Most of the cells were in the first stage, but some had reached the "spider cell stage," and rarely an ameboid cell [1] was found in the reticular portion of the degenerated area.

1. Rand, C. W., and Courville, C. B.: Arch. Neurol. & Psychiat. **27**:605, 1932.

Of unknown significance was the occurrence in a reticular area of phagocytes laden with golden yellow pigment. These macrophages were found singly or in numbers, most abundant in the region of the blood vessels.

Evidences of Attempts at Repair.—The repair of wounds of the brain is the result of a variable reaction on the part of the astrovascular system, with additional connective tissue proliferation from the meninges, particularly the dura. The wide variation in the types of reaction observed depends on the character of the lesion.

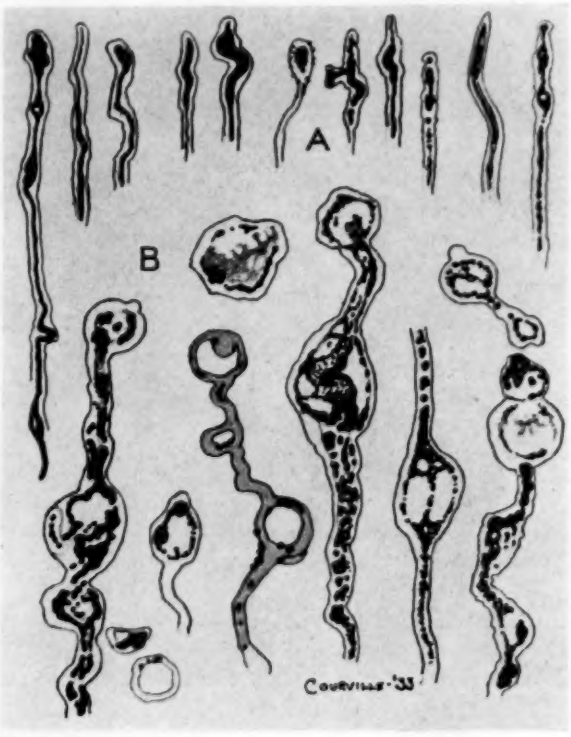

Fig. 4.—Alterations in the axis cylinders. *A* shows the types of terminations of the fibers extending into the marginal zone about the defect. *B* shows degenerating axis cylinders, illustrating the effect on the granule arrangement of the myelin swellings. Free rings and balls are also shown. Drawn from a Cajal reduced silver preparation.

Connective Tissue.—This tissue was found to be fairly abundant in the lower part of the wound of entrance and practically entirely absent elsewhere. As there was no adhesion between the brain and the dura, the connective tissue must of necessity have some other origin. It is likely that at the time of injury fragments of bone and dura were carried into the brain, resulting in serious local laceration. The degenerating brain tissue, and the presence of foreign bodies, together with extensive pial injury, evidently favored the development of a modified connective

tissue scar. The upper portion of the wound of entrance, composed of a multilocular cyst, was perhaps the site of an original local hemorrhage, which did not favor the deposit of connective tissue at its margins. The tissue about the cavity in the right temporal lobe in which the bullet was lodged gave no evidence of the formation of connective tissue.

One rather unusual feature of the connective tissue reaction was its replacement of the parenchymatous elements in the residual small fragments of cortex overlying injured white substance. When extensive scar formation had replaced the injured white matter, this replacement had been quite complete. Otherwise, this vascular reticulum was rather superficial and apparently continuous with the pia mater (fig. 5 *A* and *B*).

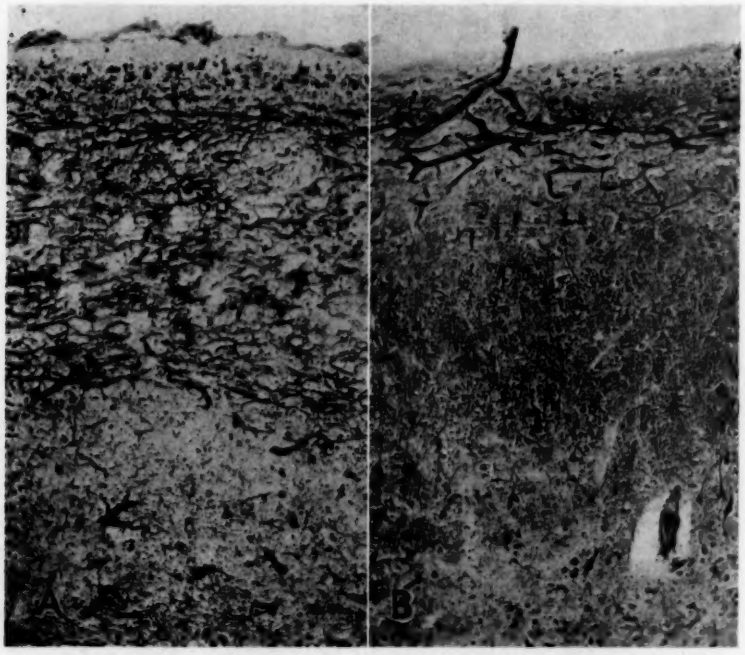

Fig. 5.—Vascular scar in the cortex. *A* shows complete replacement of the cortex by a network of blood vessels over an extensively injured white substance. *B* shows a narrow superficial vascular scar on the cortex on the opposite side of the sulcus from *A*. The underlying white substance is less seriously injured. Krajian's method for reticulum; × 100.

There was but one situation in which a combined fibro-astral cicatrix had been formed, in the lower margin of the cavitation found beneath the wound of entrance. Here was found a fairly dense scar composed of fibers arranged in parallel bundles and running at right angles to the free margin of the wound. Extending into the adjacent brain tissue was a network of blood vessels. In the deeper portions of the scar the bundles of connective tissue fibers became intermingled with those of neuroglial origin (fig. 6).

Neuroglia.—The reaction of the neuroglia in the various situations along the track of the bullet was also of interest. In the denser scar, as has already been

intimated, the neuroglia fibers were intermingled with the connective tissue fibers to form closely packed fasciculi. The cell bodies of the astrocytes were elongated and lay in the bundles of hairlike fibrils. These fibers were evidently complementary to the connective tissue fibers, for they became less abundant in the superficial connective tissue portion of the scar.

In the areas which had a more reticular appearance, surrounding the more dense central cicatrix, a more typical glial scar was found. The neuroglia formed a fairly uniformly meshed network, the fibrils forming the intervening strands while the nuclei were lodged in the interstices of the mesh.

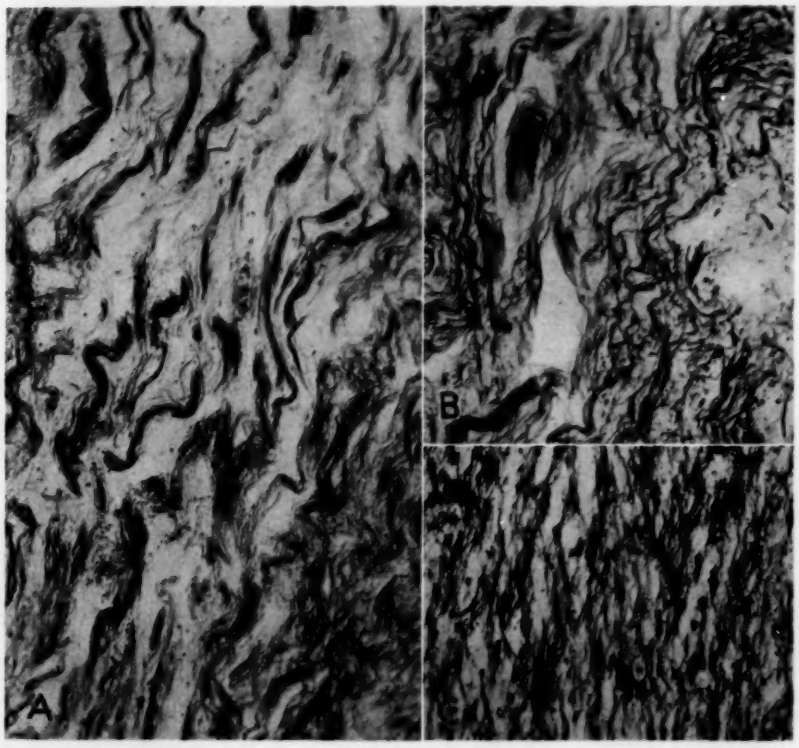

Fig. 6.—Composition of fibro-astral scar. *A* shows neuroglial and fibroglial fibers arranged in bundles. Phosphotungstic acid hematoxylin; × 160. *B* shows parallel strands of reticulin in the superficial portion of the wound. Krajian's method for reticulin; × 160. *C* shows piloidal astrocytes of Penfield, with parallel fibrils in the deeper portion of the scar. Reduced silver method; × 160.

In still other areas where the margin of the wound was more sharply defined and the adjacent tissue appeared solid well up to its borders, there was little or no neuroglial reaction. This was especially true along the upper margins of the cystlike spaces at the wound of entrance and about the cavity in which the bullet finally lodged. This variation was probably due to the amount of tissue destruction, the presence of decadent tissue encouraging the proliferation of neuroglia.

COMMENT

It is natural to assume at the outset that a wound of the brain would ultimately become quiescent, as is the case with wounds of other tissues. This study seems to indicate that this is not the case, for after an interval of twenty-two years, the processes of disintegration, of phagocytosis and probably also of repair were still taking place. Seriously damaged nerve cells had maintained their morphologic identity throughout this long interval. The peculiarities of cicatrix formation and the significance of disintegrative changes in the nerve fibers and of pigment deposit merit consideration.

The histologic changes about the wound of entrance seem to confirm Penfield's postulates as to the mode of formation and the constituents of a cerebral cicatrix. Where there has been extensive destruction of tissue in close proximity to the pia mater and especially where the dura has been injured, a marked connective tissue and neuroglial proliferation results. An intermingling of fibers of both sources forms heavy fasciculi which run at right angles to the margin of the wound. This appearance in the phosphotungstic acid preparations suggests some alternation and intertwining of the bundles of connective tissue and neuroglia fibers, resembling a coarse, irregularly woven material. The superficial position of the connective tissue and the deeper situation of the proliferating neuroglia answer the description of old wounds of the brain as given by Penfield [2] and by Foerster and Penfield.[3] The characteristic fusiform shape of the astrocytes and the arrangement of their fibers indicate their identity with the piloidal astrocytes of Penfield.[4]

In a study of the neuroglial reaction at the margins of recent wounds of the brain [5] there was found a peculiar reticular network of tissue in the interstices of which small hyperchromatic nuclei were observed. The nature of the tissue forming this mesh could not then be definitely established for it was not demonstrated in specific preparations. From the appearance of the characteristic astrocytic network of similar arrangement in a case with this long period of survival, it seems likely that the original structure was of neuroglial origin. One wonders whether the original structure was due to active regressive changes in the neuroglia consequent to the injury, or whether it was the remains of neuroglial syncytium the existence of which has been so long debated.

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2. Penfield, Wilder: Brain **50**:499, 1927.

3. Foerster, O., and Penfield, W.: Brain **53**:99, 1930.

4. Penfield, Wilder: Neuroglia, Normal and Pathological, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 455.

5. Rand, C. W., and Courville, C. B.: Arch. Neurol. & Psychiat. **27**:1355, 1932.

The occurrence of end-bulbs on the severed ends of nerve fibers was first described in detail by Cajal in a group of studies of experimental injuries of the brain.[6] These end-bulbs, recognized to be the result of regressive change rather than abortive attempts at repair, are found within a few hours after the injury, and those on the central end of the fiber may persist for months or even years. In the case under consideration, after an interval of twenty-two years, characteristic end-bulbs were not found. At the termination of the finer fibers, small irregular and at times somewhat complex expansions were observed under higher magnifications. The terminal portions of the larger fibers were slowly undergoing granular degeneration, and no end-bulbs of any sort were found. It is of interest to note in this connection that the fine fibers have been the first to reveal evidence of the formation of end-bulbs (within three hours of injury) and have been able to maintain their integrity closer to the margin of the wound. They are here discovered to retain still a residual terminal expansion, presumably a considerably modified end-bulb. This late form is probably the result of a long-continued, gradual degeneration of the original structure.

From a study of the nerve cells in the isolated cortex along the dorsal margin of the hemisphere, it has been possible to learn the fate of end-bulbs formed at the ends of fibers sectioned close to their cells of origin. In Cajal's experimental injuries of the brain, fibers sectioned in the subcortical white matter or in the deeper portions of the cortex underwent degenerative change in a retrograde direction to the last collateral where a residual end-bulb was formed. I have observed end-bulbs in this situation in the brain of a patient who survived a serious injury of the head for from six to seven months, so they may persist for at least this interval. After this there evidently takes place a slowly progressive disintegration of the residual segment of axon, for in the case at hand a short stub terminated just below the cell of origin in a "late corrosion point." The nerve cells in these instances retained their morphologic identity although no tigroid material could be identified in their cytoplasm, and their neurofibrillar apparatus had undergone granular disintegration.

A study of Cajal's experimental work leads one to believe that the end-bulbs and varicosities of the axis cylinders are coincidental with the myelin swellings. In a study of nerve fibers in recent injury of the human brain, at least one essential difference has been observed. Numerous myelin swellings of variable size and irregular distribution have been observed along the course of a nerve fiber in which no end-bulbs or varicosities were found to exist. Similarly, in consecutive

6. Ramón y Cajal, S.: Trab. d. lab. de invest. biol., Univ. de Madrid **9**:1, 39, 181 and 217, 1911.

sections stained by specific methods for myelin and for axis cylinders, in areas where end-bulbs were frequent, no myelin swellings were observed. In the case here considered, after such a long interval between the injury and death, characteristic myelin swellings were still present, while the end-bulbs and varicosities had disappeared. It seems that the swellings in the myelin sheaths occurred primarily, presumably evidence of a degenerative change in this structure, and that such swellings exerted a distorting influence on the rows of granules resulting from a breaking up of the axis cylinder.

There exists in the larger fibers a long segment of degeneration, found extending for some distance from the margin of the wound. In this zone both the myelin sheath and the axis cylinder were affected. This segment might well be designated as the "segment of late disintegration." It stands out in contrast to the finer fibers which still maintain their identity well up to the margin of the wound.

The significance of the yellowish-orange pigment contained in the macrophages scattered throughout the reticular tissue or agglutinated about the blood vessels is not entirely clear. In recent injuries their yellow color appears to be due to the presence of pigment resulting from the disintegration of red cells in the areas of local hemorrhage. While possible, it is difficult to conceive that pigment from such a source could persist for so long a time. Whether it is due to a continual process of disintegration of red cells or whether it is derived from some other source could not be ascertained.

SUMMARY AND CONCLUSIONS

Morphologically, crippled nerve cells may persist in the margins of wounds of the brain for many years. Nerve cells may, and usually do, persist in areas of cortex isolated by laceration or hemorrhage.

Even after a prolonged interval the larger nerve fibers continue to undergo regressive change at the margin of the wounds of the brain. Both the myelin sheaths and the axis cylinders are involved in this process of disintegration.

Characteristic end-bulbs were not observed in the case reported after an interval of twenty-two years. The finer fibers terminated in small filaments, spear-shaped or flame-shaped endings. When the corticifugal axis cylinders were cut off just below the cortex, the proximal portion had degenerated to within a few microns of the persisting nerve cell where it terminated in a late corrosion point.

Evidences of a persistent degenerative change were further indicated by the occurrence of free fat, yellow pigment and argentophilic material in wandering macrophages and the walls of the blood vessels, and by characteristic morphologic changes in the microglia in the adjacent cortex.

A solid cicatrix was not observed. In an area subjacent to the wound of entrance a combined connective tissue and neuroglial scar was found in which piloidal astrocytes played a conspicuous rôle. Elsewhere the neuroglial reaction was minimal and the connective tissue reaction entirely absent.

One peculiar behavior of connective tissue was its complete or incomplete replacement of the parenchymatous and interstitial elements in small portions of residual cortex over severely injured white substance.

The reticular tissue, observed in other instances within a few days after injury, ultimately comes to be formed of neuroglial elements, as indicated in the case under consideration.

ACTION OF VITAMIN D AND OF THE PARATHYROID HORMONE ON THE CALCIUM METABOLISM

AS INTERPRETED BY STUDYING THE EFFECT OF SINGLE DOSES ON THE CALCIFICATION OF DENTIN

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INTRODUCTION

Several theories have been proposed to explain the action of vitamin D and of the parathyroid hormone on the calcium metabolism. The problem is complicated by the many seemingly paradoxic phenomena which occur when the substances are administered in different dosages. A simple example illustrates this point, namely, the ability of small amounts of vitamin D to improve the calcification of bone, whereas enormous doses not only cause a removal of calcium from the skeleton but institute pathologic calcifications in the soft tissues. It is thought that experiments utilizing single doses of these substances offer distinctive opportunities for the investigation of their action, and in this connection both Laas [1] and Ham [2a] reported that there is a latent period before the onset of pathologic calcifications when vitamin D is administered in single massive doses. Ham and Portuondo [3] found that the development of the pathologic calcifications was associated with the fall of the serum calcium level after hypercalcemia had been attained. They suggested that the calcifications were caused by the inability of the serum to hold in simple solution all the calcium released from the solution maintained by the parathyroid hormone when the effect of the latter had begun to diminish.

This theory of the mechanism of calcification in hypervitaminosis D depends on the acceptance of the hypothesis which pertains to the ability

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The chemical phase of this work was prepared and written by Arthur W. Ham, the histologic phase by Isaac Schour.

1. Laas, E.: Virchows Arch. f. path. Anat. **278**:346, 1930.

2. Ham, A. W., (*a*) in Cowdry, E. V.: Special Cytology, ed. 2, New York, Paul B. Hoeber, Inc., 1932; (*b*) Arch. Path. **14**:613, 1932; (*c*) Angle Orthodontist **2**:142, 1932.

3. Ham, A. W., and Portuondo, B. C.: Arch. Path. **16**:1, 1933.

of vitamin D to increase the amount or activity of the parathyroid hormone in the circulation so that this hormone in turn attracts calcium from the various tissues of the body, particularly, the bones and intestine. Thus the hypercalcemia which develops would be caused to a large extent by the increase in the amount of the fraction of the serum calcium controlled by the parathyroid hormone. If this theory is correct, there would be less calcium available for the process of normal calcification in the body during the rise in the serum calcium level following the administration of a massive dose of vitamin D, because the shift in calcium during the rise in the serum calcium level would be from the tissues toward the blood. If, on the other hand, any of the various other theories regarding the action of vitamin D is correct, such as its supposed ability to act on the intestinal epithelium so as to allow a greater absorption of calcium, or to increase the ionization of calcium, there is good reason to believe that the process of normal calcification would undergo no change except possible improvement during the rise in the serum calcium level. This study is concerned with the investigation of the process of normal calcification during the rise and fall of the serum calcium level which ensues following the administration of single doses of either vitamin D or the parathyroid hormone. Rats were used for the experiments, and the histologic studies were made on the dentin of the growing incisor. Erdheim [4a] justly compared the sensitivity of this tissue to the drum of a kymograph. It grows continually and rapidly enough to offer definite areas as indexes of the process of calcification on separate days and consequently appears to be a unique tissue for this type of study.

MATERIAL AND METHODS

This study was based on three series of experiments.

Series C consisted of eight white rats, weighing about 110 Gm., which received one dose of 0.75 cc. of ergosterol 10,000 ×. The series included two rats which were killed at the beginning of the experiment. The remaining animals were killed at intervals of twenty-four, forty-eight and seventy-two hours following the administration of one dose (table 1).

Series Z consisted of twenty-nine adult male white rats which received one dose of 0.5 cc. of ergosterol 10,000 ×. The series included four rats which were killed at the beginning of the experiment. The remaining animals were killed at intervals of sixteen, twenty-four, forty, forty-eight, sixty-four, seventy-two, ninety-six and one hundred and thirteen hours following the administration of one dose (table 2).

Series Pa consisted of eight white rats, weighing about 165 Gm., which received one dose of 40 units of parathyroid extract-Collip by subcutaneous injection into the back. The series included two rats which were killed at the beginning of the

4. Erdheim: (*a*) Frankfurt. Ztschr. f. Path. **7**:295, 1911; (*b*) ibid., p. 175; (*c*) ibid., p. 238.

TABLE 1.—*Serum Calcium of Eight Rats That Received One Dose of 0.75 Cc. of Ergosterol 10,000 ×*

Rat	Hours After Oral Administration	Serum Calcium in Mg. per 100 Cc. of Serum
1	0	10.30
2	0	10.30
3	24	10.08
4	24	13.06
5	48	17.41
6	48	16.32
8	72	14.70
9	72	15.33

TABLE 2.—*Serum Calcium of Twenty-Nine Adult Male Rats That Received One Dose of 0.5 Cc. of Ergosterol 10,000 ×*

Rat	Hours After Oral Administration	Serum Calcium in Mg. per 100 Cc. of Serum
1	0	9.80
2	0	9.60
3	0	10.61
4	0	10.94
6	16	11.02
7	16	12.96
8	24	12.16
9	24	13.68
10	24	13.30
11	40	13.02
12	40	13.02
13	40	13.65
14	48	13.02
15	48	15.54
16	48	15.96
17	64	14.91
18	64	15.12
19	64	14.70
20	72	16.33
21	72	17.07
22	72	12.25
23	96	14.55
24	96	16.14
25	96	12.62
26	96	12.41
27	113	13.79
28	113	13.99
29	113	12.98
30	113	14.41

TABLE 3.—*Serum Calcium of Eight Rats That Received One Dose of 40 Units of Parathyroid Extract-Collip*

Rat	Hours After Subcutaneous Injection	Serum Calcium in Mg. per 100 Cc. of Serum
1	0	9.880
2	0	9.710
3	18	13.390
9	66	10.880
10	66	10.490
11	94	10.810
12	94	10.600
13	116	10.815

experiment. The remaining animals were killed at intervals of eighteen, sixty-six, ninety-four and one hundred and sixteen hours following the administration of one dose (table 3).

The animals were fed on the stock laboratory diet. A blood calcium analysis was made of each animal at the time of death (tables 1, 2 and 3) by the Collip and Clark modification of the Tisdall-Kramer method.

A histologic study was made of one or two incisors of each animal. These teeth with their investing tissues were fixed in a 5 per cent solution of formaldehyde immediately after the animal was killed. Roentgenograms were then taken, and the teeth were washed, decalcified in 5 per cent nitric acid, dehydrated, embedded in pyroxylin (celloidin) and stained with hematoxylin and eosin. The sections were cut longitudinally or transversely and mounted in serial order. Longitudinal and transverse ground sections were made of some of the teeth of the animals in series C and Pa.

CHEMICAL FINDINGS

Series C.—In the control animals the blood calcium was normal (10.3 mg. per hundred cubic centimeters of serum). In the experimental animals the blood calcium rose to 17.41 mg. as the postoperative period increased to forty-eight hours. It declined subsequently (table 1).

Series Z.—In the control animals the blood calcium was normal (from 9.8 to 10.94 mg. per hundred cubic centimeters of serum). In the experimental animals the serum calcium gradually rose to 17.07 mg. as the postoperative period increased to seventy-two hours (table 2).

Series Pa.—In the control animals the blood calcium was normal (9.71 and 9.88 mg. per hundred cubic centimeters of serum). In the experimental animals the blood calcium rose to 13.39 mg. in the animal that lived eighteen hours after the injection. It declined toward normal subsequently (table 3).

HISTOLOGIC OBSERVATIONS

Histology and Physiology of the Dentin of the Normal Rat Incisor.—Before presenting our histologic observations on the dentin of the experimental animals, we shall consider briefly the normal physiology and histology of the dentin in the incisor of the rat.

The incisor of the rat is a tooth of continuous growth, and its weekly rate of eruption in the mature animal is about 2 mm. for the upper and about 2.8 mm. for the lower tooth. Its tissues including the dentin are, therefore, formed rapidly and in the mature animal are worn down just as rapidly at the incisal edge. The dentin grows by apposition at the pulpal surface in the form of layers which move anteriorly along the long axis of the tooth and are constantly replaced by similar layers.

Thus, in the incisor of a rat killed at a given time, a given layer of dentin is situated next to the pulp or midway in the dentin substance or near the surface next to the enamel or cementum, depending on whether this stripe of dentin was laid down approximately one, twenty or forty

days before the death of the animal. The reason for this is that the incisor of the rat renews itself about every thirty-five to forty-five days. Marshall [5] found that in the mature rat the dentin grew 10 microns in twenty-four hours.

The matrix of the dentin is calcified in the form of globules that are normally small and numerous and so close together that uniformly calcified tissue results. But even in normal dentin the successive layers are not equally well calcified. Well calcified layers alternate more or less regularly and rhythmically with imperfectly calcified layers, so that there arises a stratification in the dentin, especially toward the incisal edge (Schour [6]). There is a distinct smooth boundary between the still uncalcified matrix that was laid down last, called predentin, and the calcified matrix of the dentin (fig. 1).

HISTOPATHOLOGY OF THE DENTIN OF THE EXPERIMENTAL ANIMALS

Series C.—Significant changes were observed only in the dentin formed after the injection of ergosterol. Animals C 1 and C 2, which were controls, showed normal dentin (figs. 1 and 4). Animal C 3, which was killed twenty-four hours after the injection, showed an abnormally wide layer of predentin and prominent interglobular spaces in the dentin that was calcified last, so that the boundary between the predentin and the calcified dentin was irregular (figs. 2 and 3). On the other hand, animal C 6, which was killed forty-eight hours after the injection, showed a normal picture. Animals C 8 and C 9, which were killed seventy-two hours after the injection, generally showed the following stripes in the dentin when it was traced toward the pulp: *A,* a stripe of dentin which showed a normal reaction to the hematoxylin and eosin stain and which was situated in the part of the dentin that was laid down before the injection; *R,* a layer of dentin which took a predominatingly eosin stain; *F,* a layer of dentin which took a deep hematoxylin stain, and *P,* a layer of predentin which was of approximately normal width (figs. 6 and 7).

In some fields, however, animals C 8 and C 9 showed some interesting variations and disturbances in this sequence in reference to stripes *F* and *P.* Thus, figure 12 shows the more typical arrangement, as indicated in figures 6 and 7, in the dentin on the left, but in the vertical border at the right the stripe *F* is much narrower, so that stripe *P* is abnormally wide. In figure 5 somewhat similar reaction is apparent; stripe *F* is present in the form of only a very narrow band, so the

5. Marshall: J. Dent. Research **3**:241, 1921.

6. Schour, I., in Cowdry, E. V.: Special Cytology, ed. 2, New York, Paul B. Hoeber, Inc., 1932.

layer of predentin is wider than normal. Figure 4 shows a corresponding section in the control rat C 1. Figure 11 shows an abnormally wide layer of predentin (*P*).

Series Z.—Significant changes were observed only in the dentin that was formed during the time that elapsed following the injection of ergosterol.

Animals Z 1, 2, 3 and 4, which were controls, showed normal dentin,

The changes in the experimental animals varied according to the length of postoperative life.

Practically all the experimental animals of this series showed a stripe of light, eosin-staining dentin located in a position corresponding with that of the matrix of the dentin which was laid down soon after the time of the injection. This stripe was more or less constant in width except in the most anterior portion of the teeth of the animals of longest postoperative life. In addition, in the animals that lived forty-eight hours or more after the injection, the eosin-staining stripe was followed pulpally by a stripe of deep hematoxylin-staining dentin that increased in width as the postoperative hours of life increased in number.

Thus, figure 8, illustrating the formation of dentin in animal Z 15, which lived forty-eight hours after the injection, showed the following layers of dentin when they were traced toward the pulp: *A*, normally staining dentin of preoperative history; *R*, a light stripe in which eosin predominated, and *F*, a stripe in which hematoxylin predominated. In figure 9, which illustrates the dentin in animal Z 27, which was killed one hundred and thirteen hours after the injection, one finds a similar arrangement, except for layer *F*, which was considerably wider.

Series Pa.—Significant changes were observed in the dentin formed after the injection of parathyroid extract-Collip. On the whole, the reaction of the dentin in this series was quite similar to that in series C. Animals Pa 1 and Pa 2, which were controls, showed normal dentin.

Animal Pa 3, which was killed eighteen hours after the injection, showed changes in the dentin formed last similar to those found in animal C 3 (figs. 2 and 3). Animal Pa 9, which was killed sixty-six hours after the injection, showed a normal picture similar to that of animal C 6. Animals Pa 10, 11, 12 and 13 showed the following orderly succession of stripes when they were traced toward the pulp: *A*, dentin which showed a normal reaction to the hematoxylin and eosin stain and which represents the dentin that was calcified before the injection; *R*, a layer of dentin which took a light eosin stain of fairly constant width, about 14 microns; *F*, a layer of dentin which took a deep hematoxylin stain and increased in width with an increase in the number of postoperative hours (about 40 microns in animal Pa 10 and about 70 microns in animal Pa 12), and *P*, a layer of predentin of normal width (fig. 10).

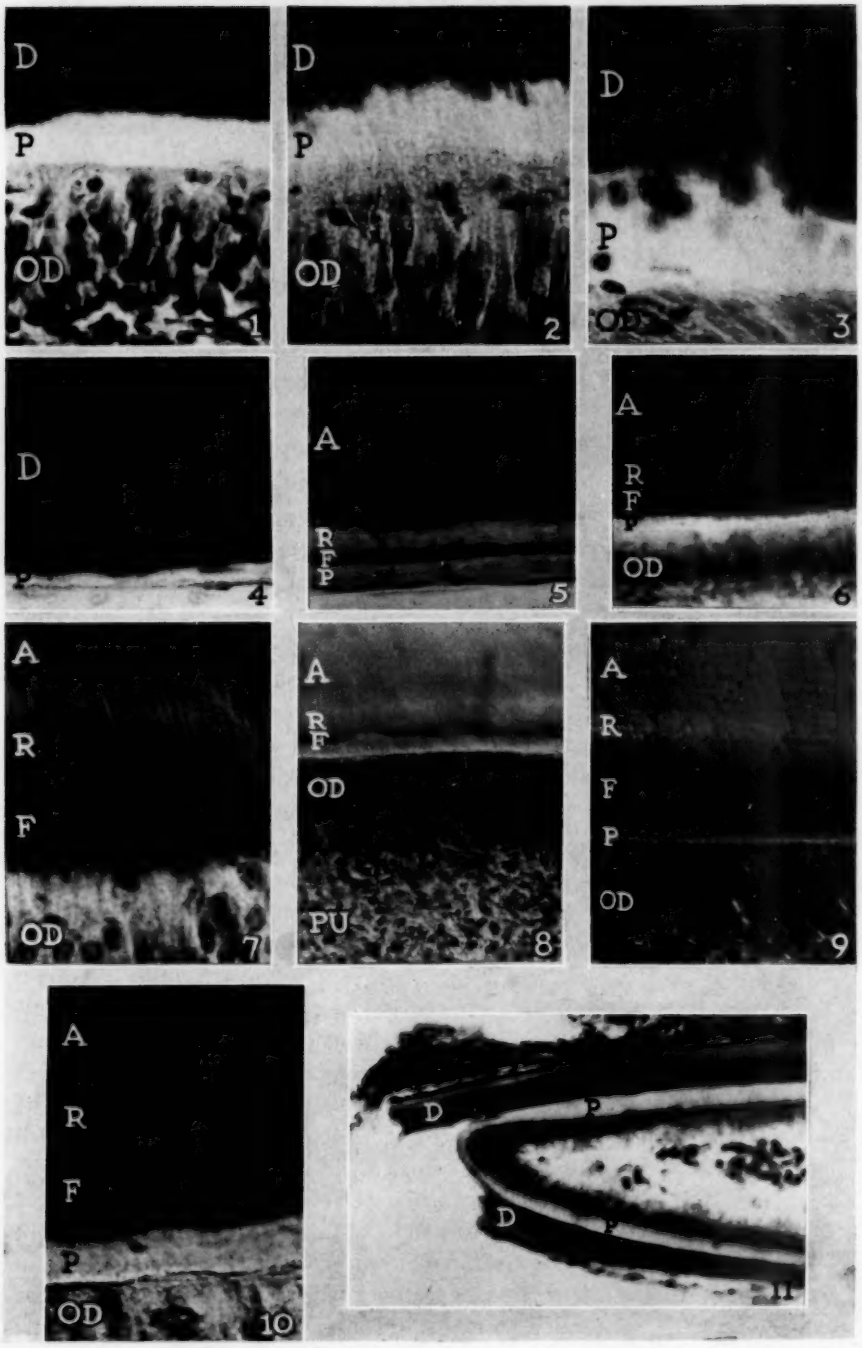
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Plate 1

EXPLANATION OF PLATE 1

Fig. 1.—Photomicrograph of a section of labial dentin of the midregion of the upper incisor of control rat C 2; × 490. *D*, calcified dentin; *OD*, odontoblasts; *P*, predentin.

Fig. 2.—Photomicrograph of a section of labial dentin of the midregion of the upper incisor of rat C 3, which was given a single dose of 0.75 cc. of ergosterol 10,000 × and killed twenty-four hours later; × 490. Note the wide layer of predentin, *P*, and compare with figure 1; *D*, dentin; *OD*, odontoblasts.

Fig. 3.—Photomicrograph of a section of labial dentin of the posterior region of the upper incisor of the same rat as in figure 2; × 490. Note the isolated globules of dentin, *D*, and the irregular boundary between the latter and the predentin, *P*; *OD*, odontoblasts.

Fig. 4.—Photomicrograph of a section of lingual dentin of the posterior region of the upper incisor of control rat C 1; × 490. *D*, dentin; *P*, predentin.

Fig. 5.—Photomicrograph of a section of lingual dentin of the posterior region of the upper incisor of rat C 8, which was given one dose of 0.75 cc. of ergosterol 10,000 × and killed seventy-two hours later; × 490. *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium and showing practically no calcification; *F*, dentin laid down during the fall of the blood calcium; *P*, predentin. Compare with figure 4.

Fig. 6.—Photomicrograph of a section of labial dentin of the upper incisor of the same animal as in figure 5; × 244. *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium and showing poor calcification; *F*, dentin laid down during the fall of the blood calcium; *OD*, odontoblasts.

Fig. 7.—Photomicrograph of a section of labial dentin of the upper incisor of rat C 9, which was given one dose of 0.75 cc. of ergosterol 10,000 × and killed seventy-two hours later; × 490. *A*, dentin laid down before the experiment; *R*, dentin laid down during the rise of the calcium curve and showing imperfect calcification; *F*, dentin laid down during the fall of the blood calcium and showing better calcification than in *A*; *OD*, odontoblasts.

Fig. 8.—Photomicrograph of a section of labial dentin of the midregion of the lower incisor of rat Z 15, which was given one dose of 0.5 cc. of ergosterol 10,000 × and killed forty-eight hours later; × 244. *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium; *F*, dentin laid down during the fall of the blood calcium; *OD*, odontoblasts; *PU*, pulp.

Fig. 9.—Photomicrograph of a section of labial dentin of the lower incisor of rat Z 27, which was given 0.5 cc. of ergosterol 10,000 × and was killed one hundred and thirteen hours later; × 244. *A*, dentin laid down and calcified before the experiment; *R*, dentin calcified during the rise of the blood calcium; *F*, dentin calcified during the fall of the blood calcium and showing more intense calcification than *A*; *OD*, odontoblasts; *P*, predentin.

Fig. 10.—Photomicrograph of a section of labial dentin of the upper incisor of rat Pa 10, which was given one dose of 40 units of parathyroid extract-Collip and killed sixty-six hours later; × 490. *A*, dentin laid down and calcified before the experiment; *R*, dentin showing poorer calcification than *A*; *F*, dentin showing better calcification than *R*; *P*, predentin.

Fig. 11.—Photomicrograph of a section of lingual dentin of the same rat as in figures 5 and 6; × 244. Note that the fracture that occurred during the dissection extends only through the calcified dentin, *D*, while the predentin, *P*, is bent. The predentin is wider than normal.

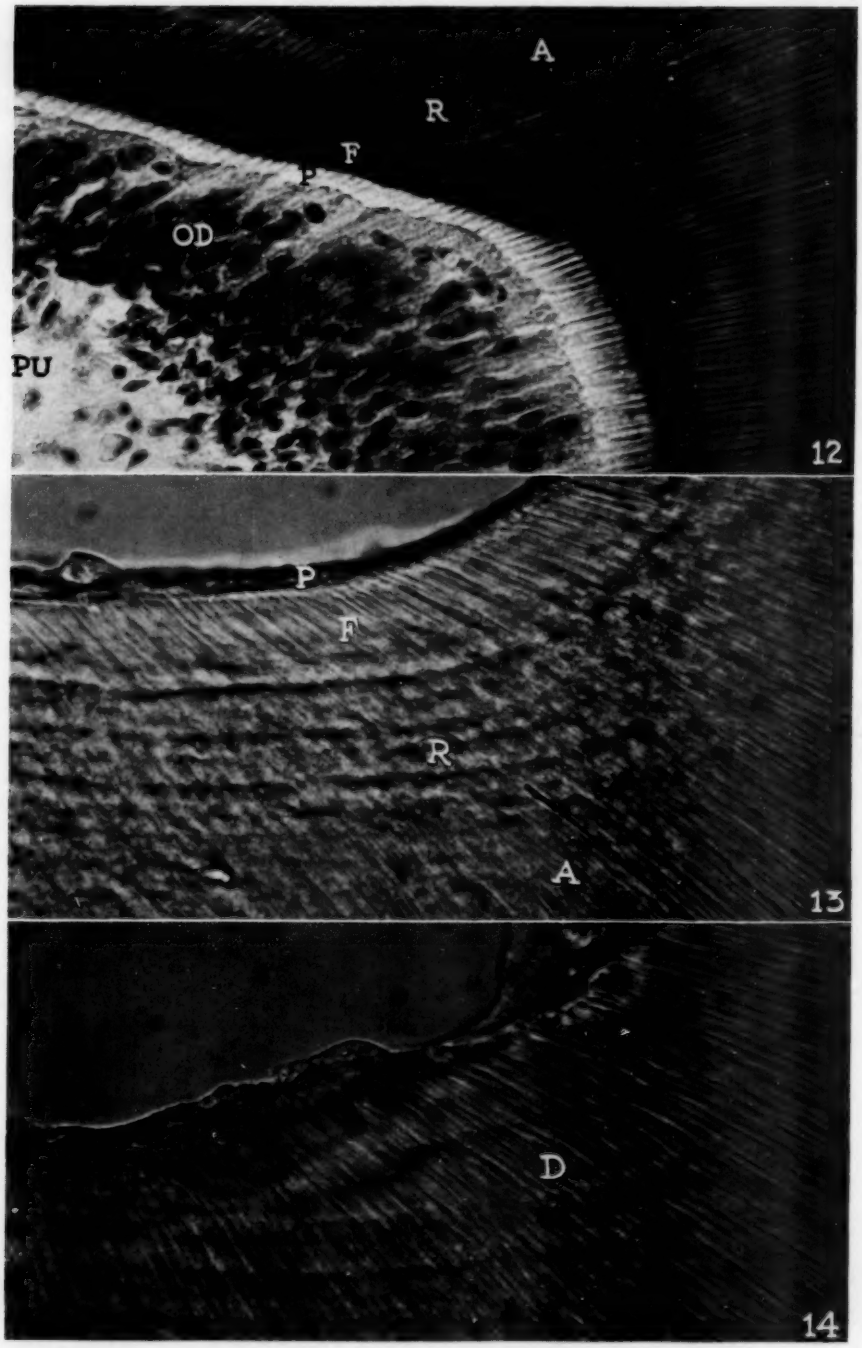


Plate 2

Explanation of Plate 2

Fig. 12.—Photomicrograph of a transverse section of dentin of the upper incisor of the same rat as in figures 5 and 6; × 490. *A*, dentin laid down and calcified before the experiment; *R*, dentin calcified during the rise of the blood calcium; *F*, dentin laid down during the fall of the blood calcium; *P*, predentin; *OD*, odontoblasts; *PU*, pulp. Note that the dentin layer, *F*, takes a more intensive stain than *A*.

Fig. 13.—Photomicrograph of a ground transverse section of dentin of the upper incisor of rat C 9, which had same experimental history as animal C 8 of figure 12; × 490. *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium; *F*, dentin laid down during the fall of the blood calcium.

Fig. 14.—Photomicrograph of a ground transverse section of dentin of the upper incisor of control rat C 1; × 490. Note normal calcification of dentin, *D*, and compare with figure 13.

Summary of the Histologic Reaction.—The histologic picture can be seen best in the experimental animals of the three series which were killed last, because these present a more complete record. Three principal types of stripes can be distinguished in the dentin of their incisors:

1. A stripe of dentin which reacted normally to the hematoxylin and eosin stain and which represents the preoperative dentin. This stripe naturally is by far the widest, as the animals were allowed to live only a relatively short time after the operation (from sixteen to one hundred and sixteen hours).

2. A stripe of dentin which took a light eosin stain and which represents the dentin that was imperfectly calcified during the rise of the blood calcium curve. This stripe was of constant width except in some of the animals of longer postoperative life, in which its most anterior portion was narrower.

3. A stripe of dentin which took a deep hematoxylin stain and which represents the dentin that was well or excessively calcified during the decline of the blood calcium curve. Its width varies directly with the length of postoperative life.

There were some incidental variations from this pattern in individual cases. Thus, in the animals of series C which had a longer postoperative life, the dentin showed localized disturbances. This may be associated with the higher dosage of ergosterol and the younger age of the animals.

COMMENT

Preliminary Histologic and Chemical Studies Made Independently.—In order to avoid the influence that the knowledge of the chemical history might have on the study of the histologic observations, the latter were first made by one of us (I. Schour) without knowledge of the blood calcium curves. The histologic study led to the conclusion that the dentin laid down soon after the injection was imperfectly calcified, while the dentin laid down later was excessively calcified. This conclusion coincided closely with the expectations that were deduced independently by A. W. Ham.

The Validity of Hematoxylin as an Indicator of Calcification.—Although we are fully aware of the limitations pointed out by Cameron [7] and Hagens,[8] in judging the state of calcification by means of hematoxylin and eosin staining, we regard the latter as a workable and dependable method for dentin for the following reasons:

1. In decalcified sections the calcified matrix of the dentin takes the hematoxylin and the uncalcified matrix takes the eosin stain, as is

7. Cameron, J. R.: J. Path. & Bact. **33**:929, 1930.

8. Hagens, E. W.: Arch. Otolaryng. **13**:824, 1931.

borne out by roentgenograms. Radiopaque dentin takes the hematoxylin and radiolucent dentin takes the eosin stain in histologic sections (Toyofuku,[9] Schour and van Dyke [10]).

2. When a fracture occurs in the dentin tissue, the former is seen in histologic sections to be limited to the dentin which takes the hematoxylin stain and does not extend into the dentin which stains with eosin. Figure 11 shows a fracture that occurred during the dissection of the upper left incisor of animal C 8. The fracture extends through the dentin which took the hematoxylin stain. In the dentin which took the eosin stain there is no break in continuity but merely a bending at an angle of about 130 degrees. The fracture occurred only in the part of the dentin which was calcified. Erdheim [4b] reported fractures in parathyroidectomized rats that extended in the enamel and in the blue-staining dentin, while "the calcium-free red-staining dentin, because it was soft and yielding, remained intact." He also reported similar observations in ribs that fractured spontaneously following parathyroidectomy.

3. Erdheim [4c] observed that, in the presence of suppuration, dentin which stained with eosin was readily invaded by pus cells, while the latter did not penetrate hematoxylin-staining dentin which was entirely surrounded by pus. He associated the lack of penetration in the blue-staining dentin with its hardness.

4. Ham [2b] observed that in pathologic calcifications produced by hypervitaminosis D the media of the coronary vessels which stained deeply with hematoxylin showed a greatly increased mineral content in the incinerated sections. He also reported that the observation of calcareous deposits in the aorta, based on the hematoxylin reaction, was substantiated in animals of similar history which were given an injection of alizarin red, and that these showed clearly, in the gross, pinkish areas in the wall of the vessel.

5. A study of ground sections of corresponding fields revealed that the stripes which in the ground sections showed good homogeneous calcification, as evidenced by the absence of interglobular spaces, corresponded to the stripes in the decalcified sections which took more of the hematoxylin stain (figs. 13 and 14).

For the reasons just stated, we feel justified in interpreting in decalcified sections a dentin stripe which stains only lightly with hematoxylin as dentin that is poorly calcified. By the same token, a stripe which stains deeply with hematoxylin represents dentin that is well or excessively calcified, depending on the intensity of its staining reaction.

Effect of the Rise in Blood Calcium.—The fact that the width of the eosin-staining stripe laid down during the rise of the blood calcium

9. Toyofuku: Frankfurt. Ztschr. f. Path. **7**:249, 1911.
10. Schour, I., and van Dyke, H. B.: Am. J. Anat. **50**:397, 1932.

level diminished in some of the animals which had a longer postoperative life suggests a partial recovery of the defect in the dentin that was laid down soon after the injection. Thus, during the fall of the blood calcium there is, as a rule, not only an improvement in the calcification of the dentin laid down at the time, but there may be, in addition, secondary calcification of the dentin previously imperfectly calcified.

Sensitivity of the Dentin Reaction.—Erdheim's statement regarding the delicacy of the reaction of dentin to changes in the calcium metabolism is fully confirmed by the observations in this study and by the unreported observations in a study made by one of us (I. Schour) in collaboration with McJunkin, Tweedy and Breuhaus.[11] The histologic study of the dentin of the incisor of the rat is found to afford much better advantages than does the study of bone in mirroring the processes that take place in the calcium reservoir. The dentin yields a delicate and a chronological record of the changes that occur during the entire duration of the experiment, provided the experiment does not last more than from four to five weeks. This suggests the possibility of using this tissue as an indicator in a biologic assay of substances that affect the calcium metabolism. The delicacy of the dentin reaction is comparable to that of the feather test used as an indicator for the female sex hormone.

THE ACTION OF THE PARATHYROID HORMONE

The literature on the extraordinarily complicated field of the calcium metabolism and the action of the parathyroid hormone has recently been comprehensively reviewed by Thomson and Collip.[12] They suggested that the hormone acts either (1) by forming very slowly, even in the presence of excess calcium, a calcium compound of parathyroid hormone, (2) by stimulating the production of an unknown calcium-binding substance, or (3) by acting primarily on the bones, with a resulting active liberation of calcium from the latter. The first two theories presume that the hormone increases the attraction of the blood for calcium, so the withdrawal of calcium from the bones would necessarily be of a passive nature. The last theory suggests direct action of the hormone not on blood but rather on bone, which results in the active liberation of calcium from the latter. Thomson and Collip [12] appeared to favor the latter theory and suggested that it is in accord with the histologic evidence which, they believed, indicates active rather than passive liberation of calcium from bone.

In the development of this theory it is obvious that a considerable amount of faith has been placed in the hypothesis which postulates that

11. McJunkin, F. A.; Tweedy, W. R., and Breuhaus, H. C.: Arch. Path. **14**:649, 1932.

12. Thomson, D. R., and Collip, J. B.: Physiol. Rev. **12**:309, 1932.

the osteoclast is an active agent in the resorption of bone. It should be remembered that this theory regarding the action of osteoclasts arose when it was believed that both the deposition and the removal of calcium was accomplished by means of specific cellular activity in bone. Recent studies in the mechanism of calcification have established that the process is of a physicochemical nature and that the cells are only indirectly concerned. In the light of this knowledge, the necessity for specific cellular activity in the process of resorption should be questioned, particularly as the evidence concerning the ability of osteoclasts in this respect has never been very convincing (Howell,[13] Arey [14]). Recently, Ham [2a, c] interpreted the osteoclast as the differentiation product of the osteogenic cell in the presence of a foreign body type of stimulus. He believed that osteogenic cells are differentiated into giant cells (osteoclasts) in the presence of breaking-down bone or cartilage in much the same manner that foreign body giant cells arise when fat is breaking down in fat tissue. In other words, he regarded them as arising primarily as the result of the disintegration of bone and cartilage. After their formation they may cause indentations (Howship's lacunae) on the surface of the bone because of the proximity of their cytoplasm to the calcified matrix. The stimulus for their origin, however, is thought to be not a need for the removal of calcium but a foreign body type of stimulus arising because of the distintegration of bone and cartilage that is already progressing.

If osteoclasts are regarded as the effect of the resorption of bone rather than as its cause, their presence in bones after the administration of parathyroid hormone offers no evidence of the direct action of the hormone on osteoclasts. Furthermore, the results of our experiments on dentin tend to disprove the theory concerning direct action on bone, as the calcification of dentin was interfered with despite the absence of osteoclasts in this region. It therefore appears that our results can best be explained by the theory which postulates that the hormone controls, directly or indirectly, a fraction of the serum calcium. Examination of the evidence for and against this theory would involve the consideration of an immense amount of controversial literature. It is discussed in detail in the reviews of Peters and Van Slyke,[15] Thomson and Collip [12] and Barr.[16] It is thought, however, that in the application of this theory sufficient care has not been taken to distinguish the changes which occur during the period of rise in the serum calcium level following a single dose of the hormone from those which occur as the serum

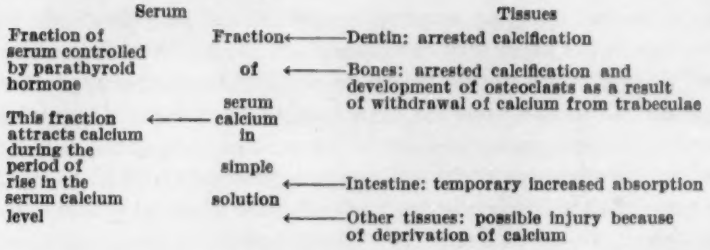
13. Howell: J. Morphol. **4**:117, 1890.

14. Arey, L. B.: Am. J. Anat. **26**:315, 1920.

15. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1931.

16. Barr, D. P.: Physiol. Rev. **12**:593, 1932.

calcium level recedes. If the parathyroid hormone acts by influencing a certain fraction of the serum calcium, a large injection of the hormone into an animal would result in calcium being attracted to the blood and, more specifically, to the fraction of the serum controlled by the hormone. The shift in calcium during the period of rise in the serum calcium level following a single dose of parathyroid hormone might be diagrammatically represented as follows:

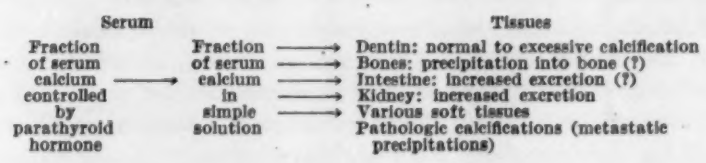


The evidence at hand which points to changes in the tissues as postulated in this diagram may be briefly summarized. Our experiments with dentin indicate that its calcification is arrested during the upswing of the serum calcium curve. Numerous investigators have shown that the bones lose calcium after administration of the parathyroid hormone (Hunter and Aub,[17] Hunter,[18] Bauer, Aub and Albright,[19] Stewart and Percival [20] and Caven and Taylor [21]). Collip showed that the hypercalcemic effects of the administration of the parathyroid hormone are intensified by the ingestion of calcium, a fact which would indicate increased absorption during the period of rise in the serum calcium level. The calcium content of the tissues during the period of rise in the serum calcium level can at present be only a matter of speculation. There is, however, some indirect evidence which suggests that the tissues may be injured during the period of rise in the serum calcium curve by virtue of the abstraction of calcium from them. In this connection, McJunkin, Tweedy and Breuhaus [11] found that injections of the hormone into the renal parenchyma caused necrosis of the tissue.

After hypercalcemia has been attained by the administration of a single dose of the hormone the calcium level begins to fall. This phenomenon may be explained by considering that the administered hormone is being eliminated, broken down or affected in some manner so that it no longer retains its former ability to attract or hold calcium.

17. Hunter, D., and Aub, J. C.: Quart. J. Med. **20**:123, 1927.
18. Hunter, D.: Lancet **1**:897, 947 and 999, 1930.
19. Bauer, W.; Aub, J. C., and Albright, F.: J. Exper. Med. **49**:145, 1929.
20. Stewart, C. P., and Percival, G. H.: Biochem. J. **21**:301, 1927.
21. Caven and Taylor, cited by Taylor, Weld, Branion and Kay.[28]

The calcium, formerly held in solution by the effect of the hormone, would then be liberated. This phenomenon would institute a shift in the equilibrium in the direction opposite to that which occurred during the period of rise, and in this phase of the process calcium might be expected to be deposited in the dentin and bones and to be excreted. There is another possibility to be considered, namely, that the release of calcium from the solution maintained by the parathyroid hormone might introduce more calcium into simple solution than could be retained without the occurrence of precipitation (Ham,[2b] Ham and Portuondo[8]). The shift in calcium which probably occurs during the fall in the serum calcium level following the hypercalcemia attained by a single dose of parathyroid hormone might be diagrammatically represented as follows:



The evidence to support this conception of the shift in calcium during the fall in the serum calcium curve is incomplete. Our work demonstrated that the calcification of dentin varied from normal to excessive during this phase. In this connection it should be again pointed out that the dentin offers a much more accurate index of the day by day process of calcification than do the bones. It is difficult to determine what picture in bone is caused by the shift in calcium during the downswing of the curve. The dentin, on the other hand, provides a new stripe of tissue for each successive day's study. The evidence for increased secretion by way of the intestine in this phase of the serum calcium curve is not yet clearcut, because most experiments in this field have been performed by administering several doses of the hormone. There is, however, good evidence to indicate that the precipitations into the soft tissues occur in this particular phase. It was shown by McLeod and Taylor[22] that the urgent symptoms of hyperparathyroidism made their appearance as the serum calcium curve showed a decided fall from its previous high level. Thomson and Collip[12] stated that "urgent symptoms of distress begin to appear at about the time when the serum calcium curve turns downward." Ham and Portuondo[8] showed that the calcifications of hypervitaminosis D occur during the period of fall of the serum calcium curve following a single dose of the vitamin. Thus there appears to be reasonably good evidence to suppose that the beginning of the fall in the serum calcium level coincides with a shift in calcium from the parathyroid-controlled fraction to one which must be

22. McLeod and Taylor: Tr. Roy. Soc. Canada (Sect. 5), 1925, p. 27.

retained in simple solution; and, furthermore, it appears that if the amount of calcium released is sufficiently great, precipitations will occur into the soft tissues.

ACTION OF VITAMIN D

Taylor, Weld, Branion and Kay [23] recently considered in detail the literature which pertains to the problem of the interrelationship of vitamin D and the parathyroid hormone. They submitted important evidence to indicate that large doses of vitamin D exert their effects through the parathyroid mechanism. Thomson and Collip [12] also discussed this problem in detail. The chief difficulty in the performance of crucial experiments in this field is the possibility of aberrant parathyroid tissue. Other theories of the action of vitamin D are not so clearcut as this one, but in general they postulate that the administration of vitamin D allows an improved absorption or utilization of calcium on the part of the affected animal. There are, of course, numerous examples in which the administration of vitamin D resulted not in an improved calcification of bone but in demineralization (Kreitmair and Hintzelmann,[24] Baumgartner, King and Page [25] and others). Grauer [26] reported on the development of osteitis fibrosa cystica following excessive and prolonged administration of vitamin D. In our experiments it was found that the calcification of dentin was arrested for a time following the administration of a single large dose of activated ergosterol. It is difficult to explain our results by any theory which postulates that vitamin D acts by simply increasing the absorption or ionization of calcium. On the other hand, our results may be explained readily if vitamin D is thought to act through the parathyroid mechanism, but only if the parathyroid hormone is thought to act on the blood by controlling a fraction of the serum calcium. Thus, by reference to the diagrams formulated to show the action of the parathyroid hormone, the calcification of dentin in relation to the serum calcium curve after the administration of vitamin D may be readily understood.

These experiments, it is thought, offer some evidence that vitamin D acts through the parathyroid mechanism. They furthermore indicate that the parathyroid hormone acts on the blood rather than on the bones. They offer evidence that, following a single dose of either substance, a shift in calcium occurs toward the blood while the serum calcium level is rising and toward the tissues while the level is falling. This

23. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: Canad. M. A. J. **24**:763, 1931.

24. Kreitmair, H., and Hintzelmann, U.: Arch. f. exper. Path. u. Pharmakol. **137**:203, 1928.

25. Baumgartner, L.; King, E. J., and Page, I. H.: Biochem. Ztschr. **213**:170, 1929.

26. Grauer, R. C.: Proc. Soc. Exper. Biol. & Med. **29**:466, 1932.

theory was utilized by Ham and Portuondo to explain the mechanism of certain metastatic calcifications, and its application allows many seemingly paradoxical phenomena encountered in studies on calcium metabolism to be explained. Confusion often arises because of the failure of investigators to appreciate the difference in the state of the serum calcium under conditions of a rising curve in contrast to conditions during a falling curve.

SUMMARY

Since the dentin of the incisor of the rat is apposed day by day, it was selected as an index of the normal calcification process as affected by the administration of single massive doses of either vitamin D or the parathyroid hormone.

Single massive doses of either substance resulted in the formation of, first, a stripe of dentin which was imperfectly calcified and, second, a stripe of dentin which was normally or excessively calcified.

The poorly calcified dentin was found to represent the area calcified while the serum calcium level was rising, and the well calcified stripe was found to represent that calcified while the serum calcium level was falling, after the attainment of a hypercalcemia.

As no osteoclasts were found in the dentin, the action of the parathyroid hormone in preventing calcification for a time did not appear to depend on its supposed ability to stimulate the active liberation of calcium from bone.

The observations could be explained by the theory which postulates that the parathyroid hormone controls a fraction of the serum calcium.

The results with vitamin D could not easily be explained by any theory which postulates that the vitamin acts by simply increasing the absorption or ionization of calcium.

The results could be explained by the theory which postulates that vitamin D acts through the parathyroid mechanism, provided the shift in calcium during the upswing of the curve is considered to be toward the blood and, during the downswing of the curve, from the blood to the bones, dentin, soft tissues and intestine.

SOME ABNORMALITIES IN RATS SUBSISTING ON DIETS POOR IN MINERAL NUTRIENTS

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During a study of the effects of diets low in salt on the rate of growth of rats, and on the proportion of mineral constituents deposited in their bones, it was noticed that the eyes of a number of the animals protruded abnormally. The proportion of inorganic matter in the skulls of all of the animals reared on these diets was so small that the bony tissue was soft to the touch; furthermore, the spines showed several curvatures, the legs were bowed and all of the bones were spongy. Various degrees of anemia were observed. The eyes themselves, although they appeared to be unusually large and distended with fluid, on histologic examination showed no signs of tissue damage from intraocular pressure, nor did the ocular muscles reveal any abnormalities. The protrusion of the eyes was not continuous; it was most marked when the animal was active, but occasionally disappeared entirely.

The effects of the realimentation of these animals with ample amounts of calcium salts or with an adequate salt mixture have been investigated.

EXPERIMENTAL METHODS

The basal ration was composed of: casein, 18 per cent; butter fat, 9 per cent; lard, 20 per cent and dried yeast, 8 per cent; the remaining 45 per cent of the ration consisted of starch. The experimental diets were made by supplying from 0.5 to 4 per cent of different salt mixtures in place of an equivalent weight of starch. In all cases 10 drops of cod liver oil was administered daily, and distilled water was furnished ad libitum. When Sure's or McCollum's salt mixtures supplied the inorganic requirements, iodine was added to the water once weekly.

The ingredients of the basal ration, exclusive of the salt mixture, furnished 1.28 Gm. of ash per hundred grams of food; of this, 0.023 Gm. consisted of calcium and 0.245 Gm. of phosphorus. Rats restricted to such a diet died in about forty days. Male albino rats, 21 days of age, of from 40 to 50 Gm. in weight, were selected for all experiments.

From the Department of Surgery, Section of Ophthalmology, Yale School of Medicine, and the Biochemical Laboratory of the Connecticut Agricultural Experiment Station.

A part of the expense of this investigation was borne by the Carnegie Institution of Washington.

A group of seventeen rats was fed on a diet that contained 0.5 per cent of Sure's salt mixture[1] and 0.28 per cent of sodium chloride. The calcium and phosphorus contents of this diet were respectively 0.063 and 0.305 Gm. per hundred grams of food. At the expiration of sixty days the femurs of nine of these rats were analyzed for ash, and the hemoglobin content of the blood was determined by a modification of the Cohen-Smith method.[2] The remaining eight rats then received a diet that contained 4 per cent of Osborne-Mendel salt mixture;[3] four rats were killed after thirty days and four after sixty days on this diet and were examined similarly.

A second group of seventeen rats was fed a diet that contained McCollum's salts[4] for sixty days, when nine were killed and examined as in the first group. To ascertain the effect of an adequate supply of calcium on the hemoglobin content of the blood, on the deposition of bone ash, and on the general condition of the animals, calcium carbonate was added to the diet of the remaining eight rats to make the calcium content of the ration equivalent to that of the ration in which 4 per cent of Osborne-Mendel salts had been included. Four animals received this ration for thirty and four for sixty days before analyses were made.

X-ray photographs were taken of four animals from each of these groups after thirty and sixty days on the diets low in salt, and also after thirty days of realimentation with a complete salt mixture or with calcium carbonate.

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1. Sure, B.: J. Biol. Chem. **58**: 681, 1924. The composition of this salt mixture is:

	Gm.
Sodium chloride	0.2022
Anhydrous magnesium sulphate	0.3117
Crystallized disodium hydrogen phosphate	0.5265
Dipotassium hydrogen phosphate	1.1158
Crystallized dicalcium phosphate (calcium hydrogen phosphate)	1.1165
Calcium lactate	0.2896
Ferric citrate	0.1385

2. Cohen, B., and Smith, A. H.: J. Biol. Chem. **39**: 489, 1919.

3. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem. **37**: 557, 1919. The composition of this salt is:

	Gm.		Gm.
Calcium carbonate	134.8	Citric acid plus water	111.1
Magnesium carbonate	24.2	Crystallized iron citrate	6.34
Sodium carbonate	34.2	Potassium iodide	0.020
Potassium carbonate	141.3	Manganous sulphate	0.079
Phosphoric acid, U. S. P.	103.2	Sodium fluoride	0.248
Hydrochloric acid	53.4	Potassium aluminum sulphate	0.0245
Sulphuric acid	9.2		

4. McCollum, E. V.; Rask, O. S., and Becker, J. E.: J. Biol. Chem. **77**:753, 1928. The composition of this salt is:

	Gm.
Sodium chloride	146.0
Anhydrous magnesium sulphate	225.0
Sodium biphosphate, U. S. P. (sodium dihydrogen phosphate) plus water	293.0
Dipotassium hydrogen phosphate	805.0
Monobasic calcium phosphate (tetrahydrogen calcium phosphate) plus water	456.0
Ferric citrate	100.0
Calcium lactate	1,098.5

For purposes of control, nine animals were fed a diet that contained 4 per cent of Osborne-Mendel salts; five of these were killed at ninety days and four at one hundred and twenty days. In addition, ten rats were fed a diet that contained 0.5 per cent of McCollum's salts and 1 per cent of calcium carbonate; five of these were killed after ninety and five after one hundred and twenty days. The hemoglobin content of the blood and the ash content of the femurs were determined. X-ray photographs of two animals from each control group were taken after sixty and ninety days on the diets.

RESULTS

Of the 34 rats fed the diets low in salt, 11 developed markedly protruding eyes; 11 developed eyes which protruded slightly; 4 developed eyes with bloody rings around them; 2 developed nervous spasms characterized by "screaming fits" in which the animals bit their tails, and 7 lost hair symmetrically from the rump and, in some cases, from the abdomen as well.

A histologic study was made of the ocular tissue and its adnexa. No definite changes were noted in the structure of the eyeball, or in the muscles that control the movements of the eye. The portion of the orbital tissue consisting of the harderian gland and of fat was normal. No signs of increased intra-ocular pressure were observed, nor was there any invasion of the ocular muscles by mononuclear cells as described by McCool and Naffziger [5] for exophthalmos in man.

Neither the pituitary nor the suprarenal glands revealed definite abnormalities; the thyroid gland, however, presented certain striking changes which are now under investigation.

The hemoglobin content of the blood, the ash content of the femurs and the A/R ratios (ratio of inorganic matter in the bones to the organic matter), as well as the gross abnormalities observed, are shown in the table.

Rats that had subsisted on diets low in salt for sixty days exhibited varying degrees of anemia as judged by the hemoglobin content of the blood. Realimentation of these rats, either by an increase in the total salts of the diet or by an increase in its calcium content, in every case resulted in an increased hemoglobin content of the blood. The level of hemoglobin in the blood was, however, never as high as that in the control rats of the same age.

The inorganic matter in the bones of rats fed diets low in salt for sixty days was greatly reduced. The feeding of adequate amounts, either of a mixture of salts or of calcium carbonate, resulted in a greater

5. McCool, J. L., and Naffziger, H. C.: Tr. Am. Ophth. Soc. **30**: 103, 1932.

Effects of Diets of Varied Inorganic Salt Content on the Blood and Bones and Also on the General Appearance of Rats

Salts in Diet, per Cent	Duration of Feeding, Days	Rat Number	Hemoglobin in 100 Cc. Blood, Gm.	Ash of Dry Fat-Free Bones, per Cent	A/R	Gross Abnormalities During First 60 Days of Experiment
0.5 Sure's salts + 0.28 sodium chloride	60	C 4764	4.8	36.1	0.57	Bloody ring around one eye
	60	C 4767	11.2	43.1	0.76	Bloody ring around one eye
	60	C 4770	13.1	44.2	0.79	
	60	C 4772	9.1	46.1	0.91	
	60	C 4777	5.0	41.4	0.70	Slight protrusion of eyes
	60	C 4779	12.3	44.5	0.80	Slight protrusion of eyes
	60	C 4781	9.7	39.5	0.65	Swollen lids and closed eyes
	60	C 4787	10.5	39.7	0.66	Slight protrusion of eyes; depilation
	60	C 4789	8.5	34.9	0.54	
Average			9.4	42.2	0.74	
0.5 McCollum's salts	60	C 4801	9.8	49.3	0.97	Protrusion of eyes
	60	C 4802	11.5	....	....	Slight protrusion of eyes; "screaming fits"; depilation
	60	C 4803	10.9	47.1	0.89	
	60	C 4806	9.1	47.2	0.89	
	60	C 4808	11.5	45.7	0.84	Slight protrusion of eyes
	60	C 4812	11.7	41.8	0.72	Slight protrusion of eyes
	60	C 4815	6.1	46.6	0.87	Protrusion of eyes
	60	C 4825	4.5	38.8	0.63	Slight protrusion of eyes; nervousness; depilation
	60	C 4826	10.9	....	....	Protrusion of eyes
Average			9.6	44.5	0.81	
0.5 Sure's salts + 0.28 sodium chloride for 60 days; then 4 Osborne-Mendel salts for 30 days	90	C 4763	13.1	61.1	1.57	Protrusion of one eye
	90	C 4765	12.9	58.9	1.43	Protrusion of eyes; bloody rings
	90	C 4769	13.1	62.5	1.66	
	90	C 4771	12.3	61.1	1.57	Bloody ring around one eye
Average			12.9	60.9	1.56	
0.5 McCollum's salts for 60 days; then 1 calcium carbonate added for 30 days	90	C 4799	14.3	61.6	1.60	Depilation
	90	C 4804	12.6	58.6	1.42	Protrusion of eyes
	90	C 4807	12.6	58.6	1.41	Protrusion of eyes
	90	C 4809	12.4	57.3	1.34	Protrusion of eyes
Average			13.0	59.0	1.44	
0.5 Sure's salts + 0.28 sodium chloride for 60 days; then 4 Osborne-Mendel salts for 60 days	120	C 4778	14.0	64.7	1.83	Slight protrusion of eyes; depilation
	120	C 4780	14.0	63.9	1.77	Slight protrusion of eyes
	120	C 4782	11.9	63.6	1.75	Malfunction of hind leg; depilation
	120	C 4784	....	64.2	1.85	Protrusion of eyes; cataract
Average			13.3	64.1	1.68	
0.5 McCollum's salts for 60 days; then 1 calcium carbonate added for 60 days	120	C 4814	13.4	63.3	1.72	Protrusion of eyes; nervousness
	120	C 4817	10.9	63.0	1.72	Bloody ring around one eye
	120	C 4819	15.0	63.6	1.75	
	120	C 4822	12.6	64.7	1.83	Slight protrusion of eye
Average			13.0	63.6	1.76	
4 Osborne-Mendel salts	90	C 4864	14.0	66.3	1.97	
	90	C 4867	15.4	66.7	2.00	
	90	C 4874	15.4	65.7	1.91	
	90	C 4876	15.0	65.2	1.87	
	90	C 4878	14.0	67.1	2.04	
Average			14.8	66.2	1.96	
0.5 McCollum's salts + 1 calcium carbonate	90	C 4880	14.0	65.5	1.89	
	90	C 4882	13.7	64.7	1.83	
	90	C 4884	13.7	65.6	1.90	
	90	C 4886	14.3	65.3	1.88	
	90	C 4888	12.6	64.8	1.84	
Average			13.7	65.2	1.87	

Effects of Diets of Varied Inorganic Salt Content on the Blood and Bones and Also on the General Appearance of Rats—Continued

Salts in Diet, per Cent	Duration of Feeding, Days	Rat Number	Hemoglobin in 100 Cc. Blood, Gm.	Ash of Dry Fat-Free Bones, per Cent	A/R	Gross Abnormalities During First 60 Days of Experiment
4 Osborne-Mendel salts	120	C 4865	15.0	67.5	2.08	
	120	C 4866	16.1	67.5	2.08	
	120	C 4875	14.0	66.1	1.95	
	120	C 4877	14.6	66.0	1.94	
Average			14.9	66.8	2.01	
0.5 McCollum's salts + 1 calcium carbonate	120	C 4879	14.0	66.5	1.98	
	120	C 4881	13.1	66.4	1.96	
	120	C 4883	14.0	66.8	2.02	
	120	C 4885	16.1	66.3	1.97	
	120	C 4889	13.7	66.8	2.02	
Average			14.2	66.6	1.99	

deposition of ash in the bones, particularly after sixty days of realimentation. The ash of the bones of such rats was in no case as high as that of the control rats of the same age.

The ratio of the inorganic matter to the organic matter in the bones (A/R ratio) was less than unity in the cases of rats that had been fed on diets low in salt for sixty days. The rise in the inorganic residues of the bones which resulted from the feeding of adequate salt mixtures or of increased amounts of calcium carbonate was, as would be expected, reflected in a rise of the A/R ratios.

The x-ray pictures of the animals that had been fed diets low in salt for thirty days revealed a moderate osteoporosis of the larger bones. The same animals, after sixty days on the same regimen, showed marked generalized osteoporosis as compared with normal animals of the same age. The animals showed a definite increase of calcification in the bony structure after realimentation for thirty days on diets containing 4 per cent of Osborne-Mendel salts or adequate amounts of calcium. The x-ray pictures indicated a greater deposition of inorganic salts in the bones of the rats after realimentation on a diet to which calcium carbonate had been added than in the bones of those animals after realimentation on the ration which contained 4 per cent of Osborne-Mendel salts. According to the chemical analyses, however, almost exactly the same degree of deposition of mineral matter was found in the bones in both groups. The x-ray pictures of the control animals revealed nothing abnormal as far as the bony structure was concerned.

The calcification of the malshapen skeleton, which occurred when adequate amounts of inorganic matter were fed, resulted in an animal with bowed legs and a curved spine. Except for these skeletal defects, after realimentation the animals looked fat, healthy and normal in every way.

SUMMARY

Rats deprived of an adequate dietary source of inorganic salts developed certain skeletal abnormalities which in some cases were accompanied by protrusion of the eyes. Histologic examination of the tissues of the eye, the suprarenals and the pituitary gland showed no striking abnormalities; apparent changes in the thyroid gland are being further investigated. A marked osteoporosis of the bones was revealed by x-ray photographs and by chemical examination of the femurs.

The realimentation of such animals with a diet that contained an adequate supply of inorganic salts or a sufficiently high level of calcium resulted in marked calcification of the bones, an increase in the hemoglobin content of the blood and a general improvement in the appearance of the animals.

UREA CLEARANCE AFTER UNILATERAL NEPHRECTOMY IN DOGS

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By the so-called urea tolerance test, Karsner, Strauss, Moore and Hanzal [1] showed that in the rabbit the removal of one kidney is followed by decreased renal function as regards elimination of urea, and that following the period of decreased capacity the remaining kidney has as great a capacity for elimination of urea as did the two kidneys. With the presentation of the urea clearance test by Möller, McIntosh and Van Slyke,[2] it was evident that the problem could be studied with greater precision and in another animal. The urea clearance in the normal dog was determined by Summerville, Hanzal and Goldblatt [3] and served as a basis for the examination of the results of unilateral nephrectomy in the dog. In this study, their methods as to care, diet and conduct of the urea clearance tests were employed. During the preparation of this paper the report by Ellis and Weiss [4] on urea clearance in man after unilateral nephrectomy appeared, but it was thought useful to place our studies on record even though the findings are essentially the same.

Mature mongrel female dogs of unknown age were used. The body weights were from 9 to 15 Kg. With aseptic precautions, under morphine and ether anesthesia, the right kidney was removed by the extraperitoneal lumbar route. Each kidney removed was studied grossly and microscopically and was demonstrated to be free from disease. At the termination of the experiments the remaining kidneys were examined, with similar results except for an increase in weight. This increase was from 42 to 48, 46 to 48, 22 to 28 and 19 to 30 Gm. respectively in animals 1, 2, 3 and 4.

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1. Karsner, H. T.; Strauss, R.; Moore, R. A., and Hanzal, R. F.: J. Exper. Med. **55**:27, 1932.

2. Möller, E.; McIntosh, J. F., and Van Slyke, D. D.: J. Clin. Investigation **6**:427, 1928.

3. Summerville, W. W.; Hanzal, R. F., and Goldblatt, H.: Am. J. Physiol. **102**:1, 1932.

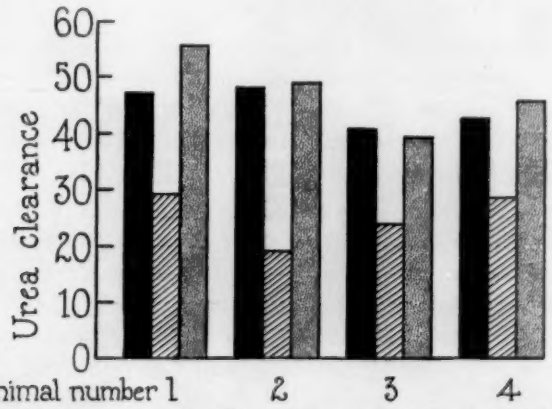
4. Ellis, L. B., and Weiss, S.: Am. J. M. Sc. **186**:242, 1933.

TABLE 1.—*Data on Dog 1**

Date	Weight, Kg.	Ideal Surface Area for Length, Sq.Mm.	Volume of Urine per Minute, Cc.	Urea in Urine, Mg. per 100 Cc.	Urea in Blood, Mg. per 100 Cc.	Clearance Corrected for Surface Area, Cc.	Comment
5/23/31	12.7	0.5879	1.53	426	17.5	62.8	180 cc. water
			0.41	850	17.5	34.4	
			0.16	1,820	17.3	46.4	
			0.14	1,590	17.3	38.6	
5/24/31	12.7	0.5879	1.60	635	28.4	60.8	317 cc. water
			0.56	1,510	28.4	50.6	
			0.20	2,800	26.8	51.3	
			0.11	3,310	26.8	45.5	
			0.13	3,050	24.0	46.2	
			0.19	3,110	24.0	61.8	
5/25/31	12.7	0.5879	1.63	250	13.7	50.7	317 cc. water
			2.23	205	13.7	69.5	
			2.92	190	13.4	70.5	
			1.38	430	13.4	75.4	
			2.47	224	12.5	75.4	
			1.97	227	12.5	59.7	
6/ 2/31	13.8	0.6091	2.18	1,830	111.5	59.9	345 cc. water;
			1.10	3,140	111.5	50.7	13.8 Gm. urea
			0.75	3,900	101.0	47.6	
			0.41	4,520	101.0	30.7	
6/11/31	14.0	0.6128	0.09	3,200	13.2	78.4	Right kidney re-
			0.05	3,200	13.2	69.0	moved 6/10/31
6/12/31	14.0	0.6128	2.76	294	11.35	116.8	350 cc. water
			0.68	517	11.35	50.2	
			0.49	740	10.4	56.8	
			0.10	2,500	10.4	79.3	
6/13/31	14.2	0.6165	5.37	210	19.4	59.2	335 cc. water
			0.43	890	19.4	31.9	
6/14/31	14.2	0.6165	1.60	1,370	110.8	32.1	355 cc. water;
			0.70	2,280	110.8	23.3	14.2 Gm. urea
			0.59	2,300	99.6	22.1	
			0.60	2,350	99.6	23.0	
			0.38	2,565	76.4	21.8	
			0.28	3,400	76.4	24.8	
6/23/31	13.6	0.6053	3.23	210	12.5	89.6	410 cc. water
			0.38	640	12.5	33.8	
			1.43	221	12.4	41.2	
7/20/31	9.2	0.5127	0.43	440	8.6	42.9	275 cc. water
			0.32	525	8.6	35.1	
			0.08	1,350	8.0	61.1	350 cc. water
			0.90	185	7.6	42.7	
			1.58	95	7.6	38.6	
7/22/31	9.2	0.5127	1.30	1,480	88.3	42.5	230 cc. water;
			0.60	2,215	88.3	29.5	9.2 Gm. urea
			0.48	2,460	73.2	31.8	
			0.23	3,610	73.2	29.8	
			0.35	2,580	55.9	34.9	
			0.20	3,705	55.9	37.5	
12/15/31	17.2	0.6678	0.73	1,205	21.6	61.1	430 cc. water; preg-
			1.30	350	21.6	32.4	nant and gave
			0.78	732	21.0	40.8	birth to 13
			0.30	1,315	21.0	33.3	pups 12/20/31
1/ 7/32	13.6	0.6053	0.18	5,180	38.5	60.4	Starved only a
			0.16	5,570	38.5	62.0	few hours
1/12/32	13.6	0.6053	4.18	1,232	143.4	59.4	340 cc. water;
			1.62	3,290	143.4	61.4	13.6 Gm. urea
			1.12	3,585	110.3	60.2	
			0.73	4,730	110.3	51.7	
			0.48	5,615	83.3	53.6	
			0.28	6,810	83.3	46.4	
1/14/32	13.4	0.6015	0.26	5,230	47.6	60.8	
			0.23	5,370	47.6	58.4	

* Only the necessary data are given. All the additional figures employed in determinations of the urea clearance,[2] such as $\sqrt{V}$, C_m and C_s, can be calculated from the material given.

The tests recorded as "plus urea" were made after the oral administration of 1 Gm. of urea in 25 cc. of water per kilogram of body weight. Control clearance values with and without urea were obtained for each dog before operation. In order to secure large volumes of urine, water was administered in some cases thirty minutes before the start of a test. Postoperative clearance values with and without added urea were obtained after one, two and three days, one, two and six weeks and six and seven months. In order to conserve space, the complete data for dog 1 only are given in table 1. In table 2 and in the chart the summarized data for the four animals are given. The clearance values have been corrected for body surface in order to compare them with the normal



Average urea clearance, corrected for surface area, in the control (solid black), postoperative (diagonal lines) and recovery (dots) periods following the administration of urea.

TABLE 2.—*Average Urea Clearance in the Control, Postoperative and Recovery Periods*

Period of Observation	Dog 1		Dog 2		Dog 3		Dog 4	
	− Urea, C/SA*	+ Urea, C/SA	− Urea, C/SA	+ Urea, C/SA	− Urea, C/SA	+ Urea, C/SA	− Urea, C/SA	+ Urea, C/SA
Control	56.2	46.9	41.1	48.0	52.1	41.6	58.8	42.7
Postoperative	57.9	29.4	37.7	19.1	37.1	23.8	35.1	28.3
Recovery	51.2	55.4	51.4	48.8	70.9	39.9	43.7	45.7

* C/SA = corrected for surface area.

values of Summerville, Hanzal and Goldblatt.[3] The advantages of this corrected value are given in their paper.

From table 2 it is evident that without the added load of urea the clearance was somewhat reduced in only two of the animals in the early postoperative period. With the oral administration of urea, however, the drop in clearance values, namely to 62.7, 39.8, 67.2 and 66.3 per cent

of the original values, is evidently significant. After recovery, the clearance values without and with the administration of urea are well within the normal range. Thus the average values for the four animals at this time are respectively 118.1, 101.6, 96 and 107 per cent of the preoperative figures.

CONCLUSION

With the urea clearance test as a criterion, unilateral nephrectomy is followed by a transient period during which the remaining kidney is physiologically deficient. After six months the remaining kidney functions as well as did both kidneys originally. Thus the enlargement of the remaining kidney of the dog is a true hypertrophy in the critical sense of the word. This result agrees with those of previous experiments on the rabbit by the urea tolerance test and with the results of Ellis and Weiss [4] in man with the urea clearance test.

AMYLOIDOSIS

EXPERIMENTAL STUDIES

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I. PRODUCTION OF AMYLOIDOSIS IN MICE

Recent work indicates that amyloidosis is not a degenerative disease but the result of a long-continued metabolic disorder, probably protein in nature. The valuable contributions of Kuczynski,[1] Smetana[2] and Jaffé[3] in the study and understanding of the pathologic process enable one to investigate this condition more adequately.

METHOD OF STUDY

Four groups of forty albino mice, one group of sixty mice and one group of thirty and one of twenty-five white mice were used. Each group was kept in separate wooden cages with false bottoms to permit the excreta to fall to the lower compartment of the cage.

The diet (stock diet) consisted of powdered whole milk and ground white bread, in proportions of 3 to 2, respectively. In addition, tap water was given.

Amyloid was produced in the manner already described by Smetana, Jaffé and ourselves,[4] consisting, briefly, in daily injections of from 0.2 to 0.4 cc. of a 5 per cent aqueous suspension of sodium caseinate for five successive days each week. The animals to be killed were given injections of from 0.5 to 1 cc. of 10 per cent aqueous solution of congo red in the right lateral tail vein. The spleen, liver and kidneys were sectioned as described.[4] Cultures were made of the sites of the injections of sodium caseinate[5] immediately post mortem, and pieces of the muscle

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2. Smetana, H.: Bull. Johns Hopkins Hosp. **37**:383, 1925.
3. Jaffé, R. H.: Arch. Path. **1**:26, 1926.
4. Grayzel, H. G.; Jacobi, M.; Maslow, H., and Warshall, H. B.: Proc. Soc. Exper. Biol. & Med. **28**:172, 1930.
5. Ushino: Verhandl. d. deutsch. path. Gesellsch. **20**:304, 1925.

were taken for histologic study. All the cultures were uniformly sterile. Sections of the muscle showed varying amounts of hyaline degeneration, fragmentation and diffuse polymorphonuclear leukocytic and lymphocytic infiltration, but no abscesses, micro-organisms or local deposits of amyloid.[3]

It has been shown—and on this point the literature has been reviewed extensively and in detail by Wickmann [6] and Schmidt [7]—that the microchemical staining reactions for amyloid vary with its age. Smetana [2] applied congo red, first suggested by Bennhold [8] as a test for amyloid intra vitam, to its pathologic study. He and others showed that this dye reveals the presence of amyloid before any other dye. This dye has therefore been used in our experiments except when noted. Application of the dye to surgical and autopsy specimens showing hyaline and granular degeneration produced none of the tinctorial pictures obtained with amyloid. When stained with congo red amyloid retains its color for about six months, slowly fading thereafter; the morphology is not affected by the loss of color. Sections must not be allowed to remain in any of the dehydrating alcohols, particularly 95 per cent, for more than from one-half to one hour, lest the red or pink-red color be removed or so weakened as to assume a purplish hue.

Until 10 or 15 injections of sodium caseinate had been given, there were no demonstrable gross or microscopic alterations. After 20 injections, the enlarged spleen was soft and more bloody and showed more sharply outlined follicles than usual. Microscopically, the sinuses, particularly the perifollicular sinuses, were tremendously distended with erythrocytes; there were no evidences of phagocytosis. Numerous giant cells appeared in the sinuses and in the red and white pulp. They were discrete, round or roughly pentagonal and measured from 15 to 25 microns. Their cytoplasm was unstained and finely granular. They contained from three to five or six well defined, round nuclei of the vesicular type, generally grouped at one pole of the cell with small amounts of finely granular chromatin scattered irregularly on a fine linin network. No cell inclusions or vacuoles were demonstrable. These cells appeared most numerous in the immediate perifollicular sinuses and red pulp. The reticular cells of this region seemed more distinct and were swollen; the cytoplasm was unstained and homogeneous. The littoral cells presented a similar appearance; the nucleus still lay near the inner border. Moderate numbers of immature polymorphonuclear leukocytes were present between the reticular cells. The lymphoid cells of the red and white pulp seemed fewer and, in the white pulp, compressed. The malpighian corpuscles were sharply outlined as small, dense masses of lymphocytes with but a faint suggestion of secondary follicles. The trabeculae and arteries showed no demonstrable changes. These changes will be referred to as precursory amyloid changes in subsequent sections.

Essentially the same appearances were present after 23, 26 and 29 injections. After the thirty-second injection the macrophages showed minute pink to pink-red crystalline or granular areas in the cytoplasm. These appeared in the central zones of the macrophages, both those within the sinuses and those in the pulp stroma. The other structures were similar to those described after 29 injections.

After 37 injections the spleen appeared smaller and less bloody. Microscopically, the macrophages contained large amounts of pink-red inclusions, which occupied the greater portion of the cell, with a narrow clear zone between these masses and the periphery of the cell, the masses appearing to lie within an intracellular

6. Wickmann, G.: Beitr. z. path. Anat. u. z. allg. Path. **13**:487, 1893.
7. Schmidt, M. B.: Verhandl. d. deutsch. path. Gesellsch. **7**:2, 1904.
8. Bennhold, H.: Klin. Wchnschr. **3**:1711, 1924.

vacuole. Such macrophages were more numerous and were arranged in linear manner, partially filling the sinuses surrounding the follicles. The reticular cells also contained similarly tinted crystalline inclusions, as did the littoral cells. The groundwork around the reticular fibers appeared thickened, those in the perifollicular zone showing pale pink-red tinting occupying short segments. The fibers themselves were unaltered. The remaining cellular, vascular and trabecular elements appeared as in the immediately preceding group.

After 50 injections the groundwork around the reticular fibers was thicker and more deeply tinted, and formed a latticework, which was most pronounced around the follicles, being gradually lost in the pulp, where small similar appearances were noted. The walls of the sinuses were outlined by a thickened, perisinus, pink-red tinted, perifibrillary groundwork, which could not be distinguished from the increased, swollen littoral cells the cytoplasm of which was filled entirely with pink-red inclusions and the nucleus of which was pyknotic. The reticular cells presented a similar appearance. These cells lay adjacent to or overlying the thickened, tinted, reticular fiber groundwork. Macrophages were numerous but fewer than in the previous sections; the outlines were indistinct, and the cytoplasm seemed to taper off into fine filaments which crisscrossed across the lumen of the sinus and were lost in the adjacent pulp. They were grouped and appeared fused in places, particularly in the perifollicular red pulp. Those within the sinuses had taken a position adjacent to the wall of the sinus, from which they could not clearly be distinguished in places. Their cytoplasm, like that of the littoral cells, was tinted uniformly a deep pink or pink-red, with a few peripheral crystalline areas. The nuclei were generally fused and had indistinct outlines and a pyknotic appearance. The sinuses were still patent but contained far fewer erythrocytes than in the preceding sections, and appeared narrowed. The follicles were small and irregular. In the outer wall of an occasional arteriole a few small pink-red crystalline granules appeared. These, however, frequently appeared within cells resembling reticular cells, so that it was impossible to say whether or not they were within the arteriolar wall.

After between 55 and 65 injections the spleen was still smaller, dry and lusterless on cut surface and without grossly visible follicles. The cut surface, in thin sections, appeared as an interlacing coarse lattice of pink-red strands of varying width between which minute pinpoint-sized nodules could be distinguished indefinitely. Microscopically, the red and white splenic pulp was composed of huge masses of homogeneous deep red substance in which appeared, irregularly, isolated lymphocytes, a few immature leukocytes and a few distorted, dense masses of lymphocytes which suggested lymphoid follicles but showed no definable structure. Numerous spaces, either narrow and slitlike or somewhat dilated, containing erythrocytes were seen in the red masses, lined only infrequently by flattened endothelial cells. Numerous macrophages, generally in agminate groups and with faint cell outlines, were seen in these masses. They blended indefinitely with the surrounding masses; their nuclei were pyknotic and showed numerous bizarre forms. The arterioles were surrounded by homogeneous pink-red masses continuous with the remainder of the red masses of the sections. Their endothelial cells were hyperplastic, and their lumens were narrowed. The dense red masses were penetrated by prominent trabeculae.

In these sections and in the immediately preceding one both the metachromasia with methyl violet and the pink when treated with iodine were obtained, the latter color changing to gray-blue or pale green-blue on the addition of sulphuric acid, as described also by Jaffé.[3] In the sections of animals receiving more than

45 injections, the metachromasia with methyl violet was shown by the intracellular and fibrillar pink-red areas tinted by vital staining. The red tinting obtained on the addition of iodine and its subsequent tinctorial changes on the addition of sulphuric acid were evidenced only faintly by the larger intracellular inclusions in the macrophages, which appeared crystalline.

The perifibrillary groundwork reticulum of the pulp and the perisinus wall and the littoral and reticular cells, which were red-tinted with the vital staining, were not tinted by the iodine. After less than about 45 injections no tinctorial changes were observed with iodine and only doubtful metachromasia with methyl violet was given by an occasional macrophage. The reticular fibers became fragmented and finally disappeared as the surrounding groundwork increased in density and depth of color.

Liver.—Like the liver of other small laboratory animals (rats, guinea-pigs and dogs), the liver of mice showed areas of cellular infiltration the significance of which is unsettled. Unlike these animals, however, these areas are comparatively infrequent in mice, are generally located around vessels in the portal field, are not sharply defined and are composed of lymphocytes and large mononuclears.[9] The Kupffer cells, while visible, are never prominent in sections from normal animals. No protoplasm is apparent as a rule, nor, normally, is there any evidence of phagocytosis.[10]

No structural changes were apparent grossly in the livers of the mice given injections throughout the experiment, nor were any demonstrable histologically until 20 injections had been given. Thereafter, until the thirtieth injection, two changes became manifest progressively in an almost quantitatively increasing manner. The Kupffer cells, previously inconspicuous and visible only as nuclei, increased in number and size, so that at the twenty-seventh injection they appeared as closely studded, rounded cells projecting into the sinusoidal lumen but still contiguous with the liver cords. Their cytoplasm was easily visible, unstained and finely granular; the nucleus, still densely basic, appeared as a well demarcated, round, eccentrically placed structure. These cells appeared most numerous in the central and midzonal portions of the lobules. Surrounding nearly every central vessel there appeared a clear, narrow, slitlike space in which were polymorphonuclear leukocytes of adult form and a few small mononuclear cells. These cellular groups—few and arranged in linear manner at the twentieth injection—increased in number, so that at the thirtieth injection they appeared as wide collars of densely packed leukocytes around the endothelium of the central vessels compressing the surrounding parenchymal cords. The endothelial cells of the central vessels at this stage appeared swollen. The sinusoids of the pericentral third of the lobules contained moderate numbers of polymorphonuclear leukocytes and an occasional small mononuclear cell, the cellular content becoming less marked peripherad. These appearances will be referred to as precursory amyloid changes in subsequent sections.

After the thirtieth injection, the cellular infiltrative changes remained unchanged until the fiftieth injection, when, as the amyloid deposits increased, the cell masses slowly and irregularly became smaller, the cells fragmented so that after 65 injections the perivascular infiltration appeared as nuclear fragments in the homogeneous perivascular amyloid, while in the adjacent sinusoids a few leukocytes were seen between the enormously enlarged and numerous Kupffer cells. At no

9. Jaffé, Rudolf: Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931, p. 233.

10. Jaffé, R. H.: Arch. Path. **2**:149, 1926; footnote 3.

stage was there any evidence of migration of any of the wandering cells through the endothelial lining of the central vessels.

After 28 injections, some of the Kupffer cells showed small crystalline areas in the cytoplasm, radially arranged and irregularly located in the cell, with a tendency to appear centrally, either unstained or having a slight brown-pink color. The nucleus was somewhat less homogeneously basic, but no distinct chromatin structure was discernible. After 32 injections, these masses had assumed a more definite pink tint. Thereafter the tint became progressively deeper and changed gradually through a pink-red to an orange-red and finally to a deep red (51 injections). Concomitantly, the crystalline masses increased in size to occupy the entire cell (47 injections), the crystalline appearance being still distinct. At this stage the central zone of the crystalline region had lost its crystalline appearance, now appearing as a homogeneous area, more deeply red. After 51 injections the greater portion of the red-tinted mass appeared homogeneous; after 56 injections the cytoplasm of the entire cell appeared homogeneous and deep pink-red.

The nucleus meanwhile had become more vesicular (41 injections) and irregular in outline (50 injections), and finally appeared in fragments (62 injections) scattered at one end of the cell or as a compressed, elliptic, vesicular but heavily chromatic, irregularly outlined body eccentrically displaced toward the liver cord surface. In this stage occasional cells with intact nuclei and with crystalline cytoplasmic inclusions appeared free within the sinusoidal lumen.

Until the fiftieth injection the outline of the Kupffer cells was distinct. Thereafter the cells appeared to fuse, so that large masses of either homogeneous red or, more infrequently, crystalline pink-red substance appeared surrounded by several flattened nuclei. These masses lay in close apposition to the cords, compressed and caused some atrophy of the cords and partially occluded the sinuses. After the sixty-fifth injection homogeneous masses of this material, in which a few nuclear fragments were scattered, completely filled many sinusoids; the atrophy of the cord in these regions was pronounced. At no time did we observe any cellular lining separating the crystalline or homogeneous tinted masses from the lumen of the sinus, nor did the nodular and linear thickenings of the capillary walls, extracellularly, appear in our sections (as reported by Jaffé [2]). The fat droplet inclusion within the Kupffer cells and the giant cell formation of the type described by Jaffé were never apparent.

After the thirtieth injection the endothelial cells lining the central vessels appeared swollen; the surrounding tissue was infiltrated with cells as described. The endothelial basement membrane seemed thickened but not pigmented or with overlying crystalline deposits. After 35 injections there appeared among these cells a few large mononuclear cells similar to those described in the spleen. After the forty-eighth injection the perivascular infiltrate contained numerous such mononuclears, their cytoplasm containing crystalline pink-red inclusions. After 51 injections there appeared homogeneous pink-red masses in this space. The endothelial basement membrane was definitely thickened and had assumed a red tint. There were, however, no crystalline masses except those within the now numerous and larger mononuclear cells, some of which appeared fused.

The extracellular homogeneous masses quickly increased in width, the discontinuous globules fusing rapidly so that in sections after the sixty-fifth injection most of the central vessels were surrounded by a zone, of varying width, of homogeneous, deep pink-red, glassy-looking substance in which were scattered nuclear fragments, a few faintly outlined mononuclears and polymorphonuclear leukocytes and a very occasional small, faintly crystalline, pink-brown mass. Near the latter one or more flattened and generally fragmented nuclei were present; no cell outlines were, however, apparent. The basement membranes could no longer

be distinguished, the red collar extending from the surrounding atrophic hepatic cords to the swollen endothelial cells, the bases of which seemed lost in the pink-red zone. No tinting or crystalline, globular, vacuolar or homogeneous inclusions could be seen in the venous lining cells.

Except for the compression and atrophic changes described in the hepatic cords, no relevant alterations were apparent in the liver cells. Even after 70 injections, we could observe no changes in the arterial walls. The cellular infiltration described by Jaffé, Maximow [10a] and others around the portal vessels showed no alterations in size, density or cellular character.

Kidney.—The kidney becomes involved relatively late. In our animals no changes were demonstrable with any regularity until 35 injections had been given,

TABLE 1.—*Degree of Amyloidosis in Albino Mice Following the Stated Number of Injections* *

Number of Injections	Number of Mice in Group						
	25	30	40	40	40	40	60
10	..	..	..	..	..	..	0
17	..	..	..	..	..	0	0
23	0	..	..	..	..	0	0
24	..	..	..	..	0	..	..
29	0	+	..	..	..	+	0
30	..	..	..	..	0	..	..
32	+	..	+	0	..	..	..
35	..	+	..	..	++	+	+
36	..	..	..	+	..	..	..
42	++	..	+	++	+	..	++
45	..	++	..	..	..	..	..
47	..	..	++	..	..	++	..
48	..	..	..	..	..	..	++
50	..	..	..	..	+++	..	..
51	+++	++	..	..	..	..	..
56	..	..	++++	+++	..	..	+++
57	..	..	..	..	+++	+++	..
62	..	..	+++	++	..	..	++++
65	..	..	..	..	++++	..	..
66	..	..	..	+++	..	++++	..
75	..	..	..	..	++++	..	..
78	..	..	..	++++	..	++++	..
89	..	..	++++	..	..	..	++++
105	..	..	..	++++	..	..	..

* 0 indicates no amyloidosis; +, early amyloidosis; ++, moderate amyloidosis; +++, moderately advanced amyloidosis; ++++, advanced amyloidosis.

when all the mice showed a moderate hyperplasia of the glomerular endothelial cells, with consequent narrowing or total occlusion of the capillary loops. In a few glomeruli these cells had lost distinct outlines, appearing as a cytoplasmic mass containing varying numbers of nuclei, their position in the glomerulus showing no regularity. No thickening of the basement membrane or tinctorial changes were apparent, except for the appearance in the renal sections of one mouse of small pink-red crystalline areas (as described in sections of the spleen and liver) in the cytoplasmic zones of the fused, giant cell-like, intraglomerular areas. In the interstitial tissue surrounding such glomeruli were small foci of lymphocytes and a few polymorphonuclear leukocytes. There were no apparent changes in the tubules, vessels or interstitial tissue.

Thereafter only the glomerular endothelial hyperplasia and the cellular infiltration of the interstitial tissue were apparent in sections obtained after 38, 42, 48 and

10a. Maximow, A. A.: Textbook of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 392.

51 injections. After 56 injections had been given, small crystalline areas were seen in glomeruli presenting the appearance of the unusual example noted after the thirty-fifth injection. In these sections a few giant cells in the interstitial tissue contained minute crystalline inclusions of a similar character, while here and there a few small, homogeneous, pink-red strands appeared among the leukocytic masses in the interstitium. These were chiefly in regions showing the macrophages.

After 65 injections—at which point this experiment terminated—there were slightly more numerous extracellular deposits, and the intraglomerular deposits were larger and surrounded by nuclei—pyknotic or even fragmented. At no stage was there any evidence of change in the epithelial cells or of vascular alteration.

Rats.—Following the technic described, seven rats were given 138 injections of sodium caseinate. They showed none of the clinical appearances associated with visceral amyloidosis. There was no evidence of any of the changes described in the organs of our mice. The frequent focal necrosis and leukocytic infiltration of the liver and the widespread diffuse and focal polymorphonuclear leukocytic content in the spleen have been described as of spontaneous occurrence. They may be an expression of spontaneous infection with Bartonella muris.[9]

COMMENT

Amyloidosis was produced in albino mice by the subcutaneous or intramuscular injection of a 5 per cent aqueous suspension of sodium caseinate. Invariably, definite evidence of amyloidosis was obtained with from 30 to 32 inoculations and became more striking and more marked with successive injections.

In the earliest stages, the amyloid appeared as radially arranged crystalline masses in the wandering macrophages and in the phagocytic fixed tissue cells, such as the littoral and reticular cells of the spleen and the Kupffer cells of the liver. After a considerably later period, similar crystals were found in the swollen, proliferated and fused glomerular endothelial cells of the kidney. As the number of injections was increased, the intracellular amyloid increased in amount. It assumed a deeper red tint. It progressively lost its crystalline character, changing to a homogeneous, glassy appearance. The first evidence of this appeared in the central portions of the deposits. Concomitantly, necrobiotic nuclear changes appeared in the cells. The cells fused. The amyloid finally appeared as homogeneous masses, showing no cellular structure. These masses became continuous with the extracellular masses that had meanwhile developed.

The final picture was one in which, depending on the number of injections, the visceral parenchyma was markedly compressed and atrophic between the masses, or was wholly replaced by the amyloid. The process made its first appearance in the spleen. Following this, the liver was involved, and then the kidney (only to a far lesser extent and after a considerable lapse of time). The process progressed at a

much more rapid rate in the spleen than in the other organs. The suprarenal glands, the intestines and the other parenchymatous organs were involved in the process.[8]

Amyloidosis can be produced in certain animals by the parenteral introduction of a variety of substances. Although in most instances the inoculating material was of protein composition, yet in some cases it was non-nitrogeneous, as, for example, silicates and selenium. However, whole protein compounds must be used, and not their intermediary or spit products. With the use of the latter, amyloidosis will not occur.[11]

Furthermore, it was the number of injections and the duration of the administration of the material, and not the quantity introduced at each injection, that influenced the pathologic process. This was pointed out by Jaffé [8] and confirmed by us. Whether 0.2 or 1 cc. of the solution was used, the rate of production of this condition was the same.

It must be added that, as will be shown in parts III and IV, diet exercises an influence on the production of amyloidosis. A thoroughly adequate diet and one containing an abundance of vitamins A and B will retard but will not prevent the development of this process. With different diets, the same animals will vary in their "time-response" to the formation of amyloid. Consequently, for comparative studies, the importance of similarity of the intake of food is obvious and has not been stressed at all.

Although there was a variability in the response of animals to the production and development of amyloidosis, yet, in our experience, all albino mice eventually acquired this disease. However, there are several species of animals in which, to date, and with present methods of production of amyloidosis, this condition cannot be produced. This difficulty was encountered by others.[12]

The mechanism in the production of amyloidosis and the origin of amyloid are as yet unknown. Several explanations have been offered. By gathering all the known data, one can construct a plausible theory which to date offers a satisfactory explanation.[13]

The injected material can be dismissed as the possible source of the amyloid, or even as one of the contributors. In the first place, the amount introduced does not influence the rate of production of amy-

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11. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 469.

12. (*a*) Lucke, B., and Markley, L. A.: Proc. Soc. Exper. Biol. & Med. **25**:642, 1928. (*b*) Robinson, B. L., and Thatcher, H. S.: ibid. **27**:580, 1930.

13. (*a*) Neuberg: Verhandl. d. deutsch. path. Gesellsch. **7**:19, 1904. (*b*) Davidson, C.: Virchows Arch. f. path. Anat. **150**:16, 1897; **155**:382, 1899; **192**: 226, 1908. (*c*) Dietl, K.: Beitr. z. Klin. d. Tuberk. **51**:18, 1922. (*d*) Letterer, E.: Zentralbl. f. inn. Med. **47**:417, 1926. (*e*) Kuczynski.[1] (*f*) Smetana.[2]

loidosis. It is wholly dependent on the number of inoculations and the period of time during which the irritant is administered. In the second place, the composition of the amyloid is independent of the chemical nature of the substance given parenterally. Different protein compounds and, more striking still, certain nonprotein compounds and inorganic substances will produce amyloidosis and the deposition of practically identical lardaceous material.[14]

That the injected substance causes an internal disorder is not questioned. Its exact nature is unknown. Perhaps it produces a disturbance of protein metabolism.

The introduction of a foreign toxic substance results in the destruction of body protein.[15] It was shown by Mendel and Rockwood [16] that the intravenous administration of edestin or casein to a dog caused the metabolism of protein which was far in excess of that ordinarily occurring in the dog plus that of the introduced protein.

It has been definitely proved by chemical analysis that amyloid is a complex protein substance.[14] Its mother substance must be of protein composition. This might be supplied from ingested protein, from "deposit protein" or from tissue protein. That an excessive and almost exclusive intake of protein might cause amyloidosis must still be considered possible in view of Kuczynski's work.[1] He, and later Smetana,[2] produced amyloidosis in albino mice by the feeding of a rich protein diet of cheese, bread, protein from chicken eggs and milk. However, it is doubtful if it can be produced so readily, if at all, in other animals.

It has been demonstrated by Voit and Landois [17] that ingested protein and deposit protein are readily metabolized, but that living tissue protein is quite resistant. This indicates that the organism is far better able to metabolize and dispose of the products of exogenous than of endogenous protein metabolism. Consequently, excessive destruction of tissue protein with liberation of such protein would place a severe metabolic burden on the animal.

That protein can circulate in the blood and be transported from one tissue to another is proved by the work of Miescher.[18] He showed that the marked development of the genital organs of starving salmon occurred as a result of the transportation in the blood of protein from the muscles.

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14. (*a*) Kravkoff, N. P.: Arch. f. exper. Path. u. Pharmakol. **40**:196, 1897. (*b*) Hanssen, O.: Biochem. Ztschr. **13**:185, 1908. (*c*) Moyeda, M.: Ztschr. f. physiol. Chem. **58**:475, 1909. (*d*) Eppinger, H.: Biochem. Ztschr. **127**:101, 1921.

15. Lusk, G.: The Science of Nutrition, ed. 4, Philadelphia, W. B. Saunders Company, 1928, p. 194.

16. Mendel, L. B., and Rockwood, E. W.: Am. J. Physiol. **12**:350, 1904-1905.

17. Quoted by Lusk,[15] pp. 80 and 90.

18. Quoted by Lusk,[15] p. 80.

However, the infiltration of amyloid in certain tissues is more than a simple precipitation or separation of circulating protein. The absence of universal deposition of amyloid in the organism, particularly at the beginning of the disease, and the constancy with which certain sites are elected suggest that some other factor or factors enter into this process. The early localization of amyloid material in the wandering and fixed cells of the reticulo-endothelial system in the spleen and liver shows an interaction which calls for an explanation. The intracellular inclusion of amyloid by the wandering macrophages and in the phagocytic fixed tissue cells of the spleen and liver suggests phagocytosis by these cells of the circulating toxic protein. For a time only intracellular amyloid is evident objectively. This may denote absence or such insignificant amounts of extracellular material that they cannot be demonstrated histochemically. With the continued progression of the process, extracellular amyloid becomes apparent, and soon fuses with the amyloid from the cells, which, about this time, undergo disintegration. The extracellular deposition of amyloid may be due to the exhaustion and inability of the fixed and wandering cells of the reticulo-endothelial system to cope with the increasing demands. Hypertrophy and hyperplasia cannot go on indefinitely. Phagocytosis cannot keep pace with the continued supply of newly liberated toxic protein. Supersaturation of this protein occurs. However, simple precipitation of the material will not explain the failure of generalized deposition of amyloid in all the crevices of the tissue and the occurrence of this deposit about the reticulo-endothelial cells. A more probable explanation may be that the exhaustion, death and disintegration of the phagocytosed cells liberate ferments, enzymes or other chemical substances which are necessary for the precipitation and deposition of the extracellular amyloid. It is probably more than a coincidence that the extracellular amyloid is observed just about the time that the phagocytic cells begin to disintegate.

This raises the question whether the latter is actually a deposit product or a homogeneous fusion of the disintegrated phagocytic cells which have lost all trace of such cellular character. To date all histologic studies fail to disprove or negate the latter possibility.

II. RESORPTION OF AMYLOID

The exact chemical composition of amyloid has not been determined. Difference of opinion still exists in regard to its constituents. Kravkoff and others [19] considered its chemical nature that of a conjugated pro-

19. Kravkoff.[14a] Friedreich, N., and Kekule, A.: Virchows Arch. f. path. Anat. **16**:50, 1859. Kühne, W., and Rudneff: ibid. **33**:66, 1865. Oddi, R.: Arch. f. exper. Path. u. Pharmakol. **33**:377, 1894.

tein, probably chondroitin-sulphuric acid. Hanssen [14b] and Henlein [20] noted an excess of sulphur in the tissues containing the amyloid material. However, when amyloid masses were obtained in purer form, i. e., devoid of the surrounding tissue, and were analyzed, no sulphur was found.[21] In addition, Eppinger [14d] showed that this complex protein substance varied in its composition and consisted of a number of different amino-acids.

The amyloid material in the tissues is an aggregate of complex protein molecules which are not always and permanently of the same composition. With its continued residence in the organs and in the presence of the factor inciting its formation, the amyloid material probably undergoes certain chemical changes and become more stable. The occurrence of this transformation is indicated by the acquisition of additional staining properties [11] and its increasing resistance to digestive ferments and to chemical changes.[22]

In man, once evident clinical signs of amyloidosis are present, the condition usually progresses and terminates fatally. As a rule, the underlying condition for the production of lardaceous changes is present. There are very few reports of cases of the disappearance of amyloidosis and recovery.[22] In all of these instances, complete and radical eradication of the suppurative focus was accomplished before the regressive changes in the amyloid occurred.

One of us (H. G. G.), with the administration of a powdered whole liver preparation, succeeded in obtaining clinical evidence of the disappearance of amyloid in the presence of active tuberculous suppuration of bone. The recession of the markedly enlarged liver and spleen in these cases, with the disappearance of other associated symptoms, was considered presumptive evidence of resorption of amyloid substance. A preliminary report of these results was made by Whitbeck.[23]

Stephanowich and Dantschakow,[24] using living bacterial cultures for the production of amyloidosis, were unable to demonstrate resorption. This, however, may have been due to the continued presence of the infection after the cessation of the injections.

Letterer [13d] did not observe resorption in amyloidosis produced experimentally by the injection of sodium caseinate.

On the other hand, Klebs and Wickmann [24] were of the belief that amyloid could be resorbed, and Kuczynski [1] noted resorption in mice.

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20. Heinlein, H.: Arch. f. exper. Path. u. Pharmakol. **149**:119, 1930.

21. Eppinger.[14d] Lucke, B.: Proc. Path. Soc. Philadelphia **42**:19, 1921. Hanssen.[14b]

22. Fox, A. R.: Brit. M. J. **1**:571, 1924. Walker, G. F.: Lancet **2**:120, 1928. Waldenström, H.: Acta chir. Scandinav. **63**:479, 1928. Schmidt.[7]

23. Whitbeck, B. H.: J. Bone & Joint Surg. **14**:85, 1932.

24. Quoted by Morgenstern.[25]

Morgenstern,[25] in his work with albino mice, concluded that resorption of amyloid occurred spontaneously following the cessation of the injections of sodium caseinate and the lapse of a sufficiently long period.

SPONTANEOUS RESORPTION OF AMYLOID

In part III we shall call attention to the retarding influence of powdered whole liver on the production and development of amyloidosis. This, coupled with the apparently favorable effect of the liver preparation on clinical cases of amyloidosis, led us to study more adequately the question of spontaneous resorption of amyloid and the rôle of the liver product on this retrogressive process.

METHOD

Two hundred and forty albino mice were placed on a stock diet of powdered whole milk, bread and water. Five successive daily injections of a 5 per cent solution of sodium caseinate were given, and were omitted during the next two days. This procedure was repeated weekly, the dose varying from 0.2 to 0.4 cc.

Six cages, each containing forty mice, were segregated for this study. After 20 injections, two cages were set aside. The mice in these two sets were no longer inoculated. One group was allowed to continue on the stock diet. To the stock diet of the other group, a powdered whole liver preparation, forming 10 per cent of the diet,[26] was added. Similarly, after 29 and 40 injections, respectively, two groups of mice were isolated.

After the cessation of the injections, two or three mice were killed at varying periods. In the case of groups 1 and 2, this method was carried out at the end of thirty, sixty-one, ninety-one, one hundred and nineteen and one hundred and seventy-five days. The mice of groups 3 and 4 were studied after twenty-eight, fifty-eight, eighty-nine, one hundred and nineteen and one hundred and sixty-one days, and the mice from groups 5 and 6 after thirty-one, sixty-three, ninety-one, one hundred and twenty-one and one hundred and forty-seven days.

In this manner we were able to study the question of resorption of amyloid at various stages in the development of the process, and to compare the influence of the diet containing powdered liver with that of the stock diet.

INTERPRETATION OF STAGES IN RESULTS

In general, we have designated as precursory changes those appearances in the splenic or hepatic sections which antedate the appearance of the intracellular inclusions and the later extracellular, red-tinted, glassy-looking substance.

Moderate amyloidosis designates the presence of larger intracellular, pink-tinted inclusions in the macrophages of the spleen. The littoral and reticular cells present similar smaller intracytoplasmic inclusions. A few thickened, pink-red, perireticular groundwork fibers may be seen in the pulp generally in regions showing the intrasinus inclusions. The liver shows crystalline, pink-red, intracytoplasmic inclusions in a few enlarged and hyperplastic Kupffer cells, and perhaps a leukocytic collar around the central vein.

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25. Morgenstern, Z.: Virchows Arch. f. path. Anat. **259**:698, 1926.

26. Osborne, T. B.; Mendel, L. B.: Washington, D. C., Carnegie Institution, 1911, no. 156, pt. 2, p. 86.

Moderately advanced amyloidosis is used to signify in the spleen larger extracellular pink-red masses deeper in color causing compression and later atrophy of the normal pulp and follicular elements. The liver shows more numerous Kupffer cell involvement and crystalline inclusions containing macrophages and extracellular red homogeneous masses among the perivascular leukocytes.

Advanced amyloidosis indicates further advance of the extracellular masses to replace the greater portion of the spleen by homogeneous red masses in which fragmented macrophages, polymorphonuclear leukocytes and lymphocytes and distorted follicles and compressed sinuses are seen. In the liver most of the central vessels are surrounded by a mass of dense, acellular, homogeneous, pink-red substance, and nearly every Kupffer cell contains large amounts of similar material. In very advanced cases small, crystalline, pink-red inclusions may be seen in the cytoplasm of fused and hyperplastic glomerular endothelial cells of the kidney.

TABLE 2.—*Resorption of Amyloid Under the Influence of the Stock Diet and a Stock Diet Containing Powdered Whole Liver* *

Group	Number of Injections	Pathologic Finding at Termination of Injection	Diet During Period of Injection	Diet During Period After Injection	Pathologic Finding at Different Periods After Cessation of Injections						
					1 Mo.	2 Mos.	3 Mos.	4 Mos.	4½ Mos.	5 Mos.	5½ Mos.
1	20	±	Stock diet	Stock diet	+	+	+	+	..	..	±
2	20	±	Stock diet	Stock diet and powdered liver	+	+	+	±	..	..	0
3	30	+	Stock diet	Stock diet	+	+	+	++	++	+++	..
4	30	+	Stock diet	Stock diet and powdered liver	±	0	0	±	0	0	..
5	40	++	Stock diet	Stock diet	++	++	++	++	+++	..	..
6	40	++	Stock diet	Stock diet and powdered liver	+	+	±	±	±	..	..

* ± indicates precursory amyloid changes; +, early amyloidosis; ++, moderate amyloidosis; +++, advanced amyloidosis.

Control animals killed after 20 injections showed only the precursory splenic and hepatic changes. After 30 and 40 injections this group presented early and moderately advanced amyloid alterations, respectively.

The results of this experiment are shown in table 2.

COMMENT

After the cessation of the injections, spontaneous resorption of amyloid in albino mice was noted only in the animals belonging to the group that had received very few inoculations and had shown only the precursory or the earliest evidences of amyloidosis (table 2). Those showing very definite signs of this condition failed to reveal any histologic evidence of retrogression. Furthermore, although the administration of the inciting agent was stopped, once definite amyloidosis set in there occurred apparently an accentuation of this process. After a varying period there was more marked and more extensive microscopic evidence of amyloidosis.

In view of the discontinuance of the injections, the more marked objective evidence of amyloidosis that was noted histologically may be due to one of two possibilities. The mere withdrawal of the external agent that provoked the destruction of tissue protein does not necessarily cause an immediate cessation of the morbid process. The latter, once initiated, may continue for some time and thus increase the amount of amyloid in the tissue. Another explanation may be offered. The material contributing toward the formation of amyloid undergoes chemical change during its residence in the tissue. With successive transformations it becomes more visible or can be more readily demonstrated by the present methods of staining.

On the other hand, after the inoculations were stopped, resorption of amyloid in early and in moderate cases of amyloidosis was observed in albino mice that were given a diet to which a preparation of powdered whole liver had been added. Moderately advanced and advanced cases of amyloidosis failed to show any evidence of retrogression of this process.

As mentioned, the amyloid material undergoes chemical change in the tissue, and apparently becomes increasingly more stable and more resistant to disintegration and decomposition. This probably accounts for the failure of spontaneous resorption except in the earliest stages, and of resorption following the use of the liver product in the animals in which the changes progressed to the stage of moderately advanced amyloidosis.

The favorable influence of the powdered whole liver on resorption may be due to the presence of an active potent principle in the liver, or to its abundant supply of reticulo-endothelial cells with its chemical products. The probable important rôle of the reticulo-endothelial system with its fixed and wandering cells in this pathologic condition was described in part I.

Whatever may be the final explanation, there is a retarding influence of the powdered whole liver on the formation and production of amyloidosis.[12a] Resorption is accelerated by liver once the injections are discontinued, provided that advanced amyloidosis has not set in.

III. INFLUENCE OF DIETARY INGREDIENTS AND INGESTED CHEMICAL SUBSTANCES ON THE PRODUCTION OF AMYLOIDOSIS

Since the basic nature of this disorder is metabolic, qualitative differences in diet may exercise an influence on the disease. Consequently, the rôle of diet, certain chemical substances, a desiccated whole liver preparation and two specific fractions of the liver was investigated.

Kuczynski [1] was the first to describe the production of amyloidosis by the feeding of a diet consisting almost exclusively of cheese. This

was confirmed by Smetana [2] but not by others.[27] Jaffé [3] noted the retardation of the production of amyloidosis by the addition of small amounts of cholesterol to the diet. Except for these studies, investigators in this field did not concern themselves with the rôle of diet. In fact, they did not even mention the nature of the food which was given the animals.

METHODS AND RESULTS

Thirteen different studies were carried out (table 3). The same procedure was employed as outlined in part I.

1. *Stock Diet.*—The results in these groups of mice are described in part I.

2. *Adequate Diet.*[28]—This group consisted of sixty mice. The diet included: yellow cornmeal, 64 Gm.; linseed meal, 10 Gm.; commercial casein, 6 Gm.; ground alfalfa, 2 Gm.; sodium chloride, 9.5 Gm.; calcium carbonate, 0.5 Gm.; whole powdered milk, 15 Gm., and cod liver oil, 2 Gm.

Pathologic studies were carried out after 33, 42, 47, 56, 62, 80 and 89 injections of sodium caseinate.

No changes were apparent until after 62 injections, at which time the spleen showed early evidences of amyloidosis. The liver and kidneys remained unaltered.

After 81 injections moderate amyloidosis set in. This condition became more marked with an increasing number of inoculations.

3. *Stock Diet Plus Powdered Whole Liver.*[29]—Two groups of forty mice each were placed on the stock diet to which was added powdered whole liver, the latter forming 10 per cent of the total diet. All of them were given injections of 5 per cent suspension of sodium caseinate according to the routine procedure.

Two mice were killed at successive intervals after 32, 42, 56, 62, 66, 78 and 105 injections. After 66 injections early evidences of amyloidosis were noted.

In this group no histologic alterations were apparent in the spleen until after the forty-second injection. At this time the spleen showed the appearances noted in group 1 before the thirty-second injection. After 56 injections the appearance was that of the spleen of group 1 at the thirty-second injection stage, while not until 66 injections had been administered did definite amyloid, in amounts approximately equal to that seen in group 1 after the forty-second injection, appear in the spleen.

Likewise, no hepatic changes were apparent until after the fifty-sixth injection, when the appearance was that observed in animals of group 1 after the thirty-second injection. After 66 injections had been given, the sections of the liver presented the picture of animals of group 1 after 42 injections. No renal changes, except for a moderate glomerular endothelial cell hyperplasia, were apparent even after the sixty-sixth injection.

4. *Stock Diet with the Addition of Ground Beef Meat.*—To the stock diet ground beef meat, forming 10 per cent of the total diet, was added and fed to a

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27. Strasser: Ztschr. f. d. ges. exper. Med. **36**:381, 1923. Morgenstern.[25]

28. The diet is based on the recommendation of H. Steenbock (Science **58**:449, 1923) for a well balanced diet.

29. The preparation of desiccated whole liver is so made that 1 Gm. is obtained from 8 Gm. of raw fresh liver.

group of forty albino mice. The inoculations with 5 per cent nutrose suspension were performed in the usual routine manner. After 32, 36, 42, 56, 62, 66, 78 and 105 injections, respectively, two mice were killed and studied.

Amyloidosis was noted after 32 injections, and with successive injections increasing amounts of amyloid was observed.

The findings here corresponded to those obtained in group 1 (stock diet).

5. *Stock Diet with the Addition of Liver Extract No. 343.*[30]—Fifty albino mice were placed in this group. The diet consisted of 1 part of powdered liver extract

TABLE 3.—*Influence of Diet and Dietary Factors and Ingested Chemical Substances on the Production of Amyloidosis* *

Number of Injections	Stock Diet	Adequate Diet	Stock Diet and Liver Meal	Stock Diet and Ground Beef	Stock Diet and Liver Extract No. 343	Stock Diet and Liver Extract with Ferrous Ammonium Citrate	Stock Diet and Cholesterol	Stock Diet and Sodium Acid Phosphate	Stock Diet and Sodium Bicarbonate
11	..	..	..	..	..	0	..	..	..
15	0	..	..	..	..	..	..	0	..
18	..	..	..	..	..	..	..	..	..
20	0	..	..	..	..	..	..	0	..
21	..	..	..	..	..	..	..	0	..
23	..	..	..	..	..	0	..	..	..
24	..	..	..	..	0	0	0	..	..
27	..	..	..	..	..	..	..	..	0
30	..	..	..	..	..	..	..	..	..
31	..	..	..	..	0	..	0	..	..
32	+	..	0	+	..	..	..	..	..
33	..	0	..	..	..	..	..	..	..
35	..	..	..	..	..	..	..	..	..
36	++	..	..	+	..	..	++	..	..
39	..	..	..	..	0	..	..	..	..
42	++	0	0	++	..	..	..	..	..
43	..	..	..	..	..	..	++	..	..
46	..	..	..	..	+	..	..	..	..
47	..	0	..	..	..	..	..	..	..
50	..	..	..	..	..	..	..	..	..
56	+++	0	±	++	++	..	..	..	..
57	..	..	..	..	..	..	..	..	..
62	..	±	±	+++	..	..	..	..	..
66	..	..	+	++++	..	..	..	..	..
78	..	..	+	++++	..	..	..	..	..
80	..	+	..	..	..	..	..	..	..
89	..	++	..	..	..	..	..	..	..
105	..	..	++	++++	..	..	..	..	..

* 0 indicates no amyloidosis; ±, precursory changes of amyloidosis; +, early amyloidosis; ++, moderate amyloidosis; +++, moderately advanced amyloidosis; ++++, advanced amyloidosis.

no. 343 and 24 parts of the stock diet. Two or three mice were killed after 24, 31, 39, 46 and 56 injections.

No amyloidosis was noted after 24, 31 and 39 injections. Following the forty-sixth injection, there were moderate amyoid changes in the spleen and early amyloid changes in the kidneys.

6. *Stock Diet with the Addition of Liver Extract with Ferrous Ammonium Citrate.*[31]—Fifty albino mice were placed in this group. One part of liver extract with ferrous ammonium citrate to 29 parts of the stock diet was fed to the mice. Injections of a 5 per cent solution of sodium caseinate were given in the usual manner.

30. One gram of this preparation is prepared from 25 Gm. of raw fresh liver.

31. One gram of this preparation is prepared from 30 Gm. of raw fresh liver and 0.17 Gm. of ferrous ammonium citrate.

In these two studies, approximately equivalent amounts of the products obtained from raw fresh liver were added to the stock diet.

The mice in this group fared badly. A rapid decline in their health occurred, as evidenced by their appearance, behavior and appetite, and by the high mortality. Three mice were killed after 11 injections, and two mice after 24 injections. After the latter period, none survived. The killed mice showed no evidence of amyloidosis.

It was decided to repeat this experiment with another group of forty mice, in order to study the possibility of infection or some other unknown factor as a cause of the early death of the mice. However, the results were the same. After 23 injections, only two mice were alive, and these showed no amyloidosis when killed.

7. *Stock Diet with the Addition of Cholesterol.*—A diet consisting of 0.8 Gm. of powdered cholesterol (Eimer and Amend) to 100 Gm. of the stock diet[32] was fed to a group of fifty mice. The mice were killed after 24, 31, 39 and 46 injections.

There was no evidence of amyloidosis after 24 and 31 injections. Following the thirty-ninth injection moderate splenic amyloid and slight perivascular intracellular hepatic amyloid were observed. After the forty-sixth inoculation moderate perifollicular and intrapulp splenic amyloid and slight perivascular intracellular hepatic amyloid could be demonstrated.

8. *Stock Diet with the Addition of 1 or 2 Per Cent of Sodium Acid Phosphate.*—Two groups of forty mice each were given the stock diet, with the addition of sodium acid phosphate, one set receiving 1 per cent of the total food in the form of the acid salt, the other 2 per cent. In the former group, none of the mice survived longer than twenty-six days on this diet, during which time 20 injections were given; in the latter group all were dead after 15 injections and a period of twenty-one days on the diet. None of the mice killed after 15 and 20 injections showed evidences of amyloidosis.

A control group of twenty uninoculated mice were placed on the diet containing 2 per cent of the acid salt. These mice apparently thrived on this diet. At the end of a period of two hundred days, they were still alive.

The urine was repeatedly tested with litmus and was found acid.

9. *Stock Diet with the Addition of 1 or 2 Per Cent Sodium Bicarbonate.*—Two groups of forty mice received the stock diet, which contained sodium bicarbonate. In the case of one group, 1 per cent of the alkali salt was added to the diet; in the second group, 2 per cent of the salt. None of the mice survived more than 27 injections or after receiving the diet for thirty-six days. The urine became alkaline to litmus within seven days. The control group of twenty mice, which did not receive the inoculations, still lived at the end of two hundred days.

No evidence of amyloidosis was noted in the mice that were killed.

COMMENT

On the ordinary stock diet, definite amyloidosis was produced in all albino mice after from 30 to 35 injections. The condition became progressively more marked with the increasing number of injections.

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32. Cholesterol was added in amounts approximating those used by Jaffé[3] in order to repeat his work and to compare its influence with the substances utilized in our investigation.

On a highly adequate diet, the mice failed to show any evidences of amyloidosis until they had received 62 injections. Furthermore, it was only after 81 injections that they showed definite signs.

This demonstrates the highly preventive character of such a diet, and the important rôle that diet may play in this disease. It is possible that the failures of some of the investigators in the production of amyloidosis in albino mice and in other animals may have been due to their lack of consideration of the rôle of dietary factors.

The addition of powdered whole liver to the stock diet also caused significant retardation. The first suggestive signs of amyloid formation appeared after the fifty-eighth injection. Such an effect was not obtained when ground beef meat, liver extract no. 343, liver extract with ferrous ammonium citrate or cholesterol was included in the stock diet in place of the desiccated whole liver.

That the marked delay in the formation of amyloid in mice receiving the powdered whole liver is not due to the inclusion of animal meat or protein in the diet is evidenced by the absence of such protection in the mice receiving the addition of ground beef meat. Consequently, it is due to some other factor or factors in the whole liver, which, furthermore, are not present in liver extract no. 343 and liver extract with ferrous ammonium citrate. The rich vitamin content of liver may be the determining factor, in view of the retarding influence of vitamins A and B (part IV). Another possibility is the presence of an active principle in the liver. The possible manner in which the liver exerts its beneficial effect is discussed in part II.

The effect of the addition of a weak acid or base to the stock diet cannot be studied satisfactorily in view of the early death of the mice. However, such additions do not accelerate the formation of amyloid, but in conjunction with the inoculations seem to impair the health of the animals.

IV. INFLUENCE OF VITAMINS ON THE PRODUCTION OF AMYLOIDOSIS

The possibility that the qualitative nature of the diet might influence this process was considered worthy of study. In the preceding section evident retardation of the development of amyloidosis was noted with certain diets. Furthermore, delay in the production of amyloidosis occurred in the group whose stock diet included desiccated powdered whole liver. Ordinarily, on the so-called stock diet, which consisted of powdered whole milk and finely ground white bread, definite amyloidosis set in after from 30 to 35 injections of a 5 per cent suspension of sodium caseinate. With the diets contributing toward the retardation of the condition, amyloidosis did not appear until much later, sometimes not until after 66 injections. Each of the diets mentioned had one thing in common. They all contained an abundance of vitamins A, B

complex and D. The present investigation was undertaken with the object of determining if a single vitamin or a specific combination of them was responsible for the effect.

METHODS AND RESULTS

The experiment was carried out in two parts. The first consisted of the use of a synthetic diet with the addition of single vitamins and various combinations of them. In the second study, the vitamins were added to the stock diet consisting of powdered whole milk and ground white bread.

Part A: Synthetic Diet and Vitamins.—Sixteen groups of albino mice were formed, each containing thirty animals. The synthetic diet was composed of 31 per cent commercial casein, 40 per cent corn-starch, 22 per cent lard and 7 per cent salt mixture.[33] The diets fed the group were as follows:

(*A*) Synthetic diet [34]

(*B*) Synthetic diet plus 10 per cent butter fat (vitamin A)

(*C*) Synthetic diet plus 5 per cent powdered yeast (vitamin B)

(*D*) Synthetic diet plus orange juice (60 cc. per 100 cc. of tap water) (vitamin C)

(*E*) Synthetic diet plus 1 per cent viosterol (vitamin D)

(*F*) Synthetic diet plus butter fat plus yeast (vitamins A and B)

(*G*) Synthetic diet plus butter fat plus orange juice (vitamins A and C)

(*H*) Synthetic diet plus 5 per cent cod liver oil (vitamins A and D)

(*I*) Synthetic diet plus butter fat plus yeast plus orange juice (vitamins A, B and C)

(*J*) Synthetic diet plus cod liver oil plus yeast (vitamins A, D and B)

(*K*) Synthetic diet plus cod liver oil plus orange juice (vitamins A, D and C)

(*L*) Synthetic diet plus yeast plus orange juice (vitamins B and C)

(*M*) Synthetic diet plus yeast plus viosterol (vitamins B and D)

(*N*) Synthetic diet plus yeast plus viosterol plus orange juice (vitamins B, C and D)

(*O*) Synthetic diet plus viosterol plus orange juice (vitamins C and D)

(*P*) Synthetic diet plus cod liver oil plus yeast plus orange juice (vitamins A, B, C and D)

The routine procedure for the production and study of amyloidosis which was described in part I was carried out. The animals were killed and studied at intervals as shown in table 4. The duration of the study was limited by the failure of the animals to survive when subjected to injections of sodium caseinate suspension while on the synthetic diet. Some of the groups succumbed so rapidly that these phases of the experiment were repeated.

Part B: Stock Diet and Vitamins.—The failure of maintenance of mice on the synthetic diet for a sufficient period of time for satisfactory study of the influence of vitamins on amyloidosis led to a similar study on mice given what is apparently

33. Mitchell, H. S., and Mendel, L. B.: Am. J. Physiol. **58**:211, 1921.

34. The diet is based on the standard diets for mice described by Mitchell and Mendell [33] and by Beard (Am. J. Physiol. **75**:645, 1926).

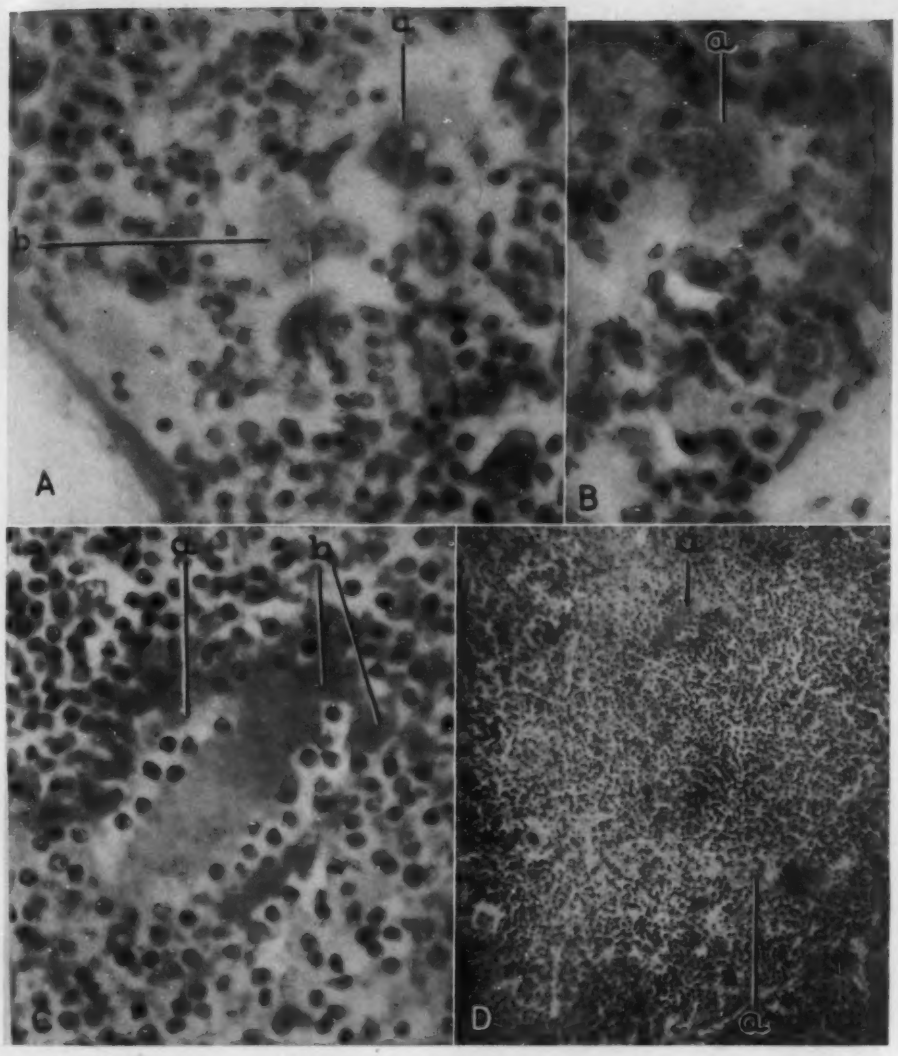

Fig. 1.—Microscopic appearance of the spleen after injections of sodium caseinate. *A*, the spleen after 36 injections of nutrose, showing 4 uninucleated (*a*) and 1 multinucleated macrophage (*b*) in the pulp sinus. The cytoplasm of the former was tinted deep pink-orange, that of the latter deep red. The latter cell shows poorly defined cellular and nuclear outline and some nuclear fragmentation. This stage just precedes the first appearance of extracellular amyloid; × 400. *B*, the spleen after 46 injections of sodium caseinate, showing a much enlarged, poorly outlined, multinucleated macrophage (*a*) in the splenic pulp. The cell outline can clearly be seen only in a few places; elsewhere along the periphery, it merges with adjacent thickened reticular groundwork amyloid fibers; × 400. *C*, the spleen after 54 injections of sodium caseinate, showing a mass of homogeneous amyloid within a pulp sinus, distending it and fusing at its upper end with an adjacent thickened sinus wall (*a*). Two very faintly outlined macrophages (*b*), their nuclear outlines only visible, can be seen in this zone; × 400. *D*, the spleen after 42 injections of sodium caseinate, showing thickened strands (*a*) of reticular groundwork fibers in the perifollicular zone (fig. 2*A*). These strands, as well as those in the following photomicrographs, assumed the specific pink-red to red tint with congo red; × 80.

TABLE 4.—*Summary of Pathologic Findings in Albino Mice on Synthetic Diet and Combinations of Vitamins* *

Number of Injections	Synthetic Diet (Group A)	Synthetic Diet Plus: Vitamin A (Group B)	Vitamin B (Group C)	Vitamin C (Group D)	Vitamin D (Group E)	Vitamins A and B (Group F)	Vitamins A and C (Group G)	Vitamins A and D (Group H)	Vitamins A, B and C (Group I)	Vitamins A, B and D (Group J)	Vitamins A, C and D (Group K)	Vitamins B and C (Group L)	Vitamins B and D (Group M)	Vitamins B, C and D (Group N)	Vitamins D and C (Group O)	Vitamins A, B, C and D (Group P)
18	0	±	0	..	0	..	..	0	..	..	0	..	0	..	..	..
20	..	..	..	..	..	..	..	0	..	..	..	..	..	++	0	0
21	..	..	..	..	..	..	..	..	..	0	0	0	0	..	..	..
23	..	..	..	0	..	0	0	..	0	..	..	..	..	..	..	..
24	0	+	0	..	..	..	..	0	..	..	..	..	..	..	0	0
25	..	..	..	..	..	..	..	..	..	+	0	..	..	..	..	..
26	..	..	..	..	..	..	..	..	0	..	..	0	..	..	..	..
27	..	..	..	..	..	..	..	..	..	..	..	..	0	..	0	0
31	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	..
33	..	..	..	..	..	..	±	..	..	..	..	..	..	..	..	0
34	..	++	..	..	..	..	..	..	..	..	..	..	..	..	..	..
36	..	..	..	..	..	0	..	..	..	..	..	..	..	..	..	..
42	..	..	..	..	..	0	..	..	..	..	..	..	..	..	..	..
45	..	..	..	..	..	..	..	..	0	..	..	..	..	..	..	..
53	..	..	..	..	..	..	..	..	0	..	..	..	..	..	..	..
67	..	..	..	..	..	..	..	..	0	..	..	..	..	..	..	..

* 0 indicates no amyloidosis; ±, doubtful amyloidosis; +, early amyloidosis; ++, moderate amyloidosis.

TABLE 5.—*Summary of Pathologic Changes in Albino Mice on Stock Diet and Combinations of Vitamins* *

Number of Injections	Stock Diet (Group A)	Stock Diet Plus Vitamin A (Group B)	Stock Diet Plus Vitamins A and D (Group C)	Stock Diet Plus Vitamin D (Group D)	Stock Diet Plus Vitamin B (Group E)	Stock Diet Plus Vitamins A and B (Group F)	Stock Diet Plus Vitamins B and D (Group G)	Stock Diet Plus Vitamins A, B and D (Group H)
24	..	0	0	+	0	0	+	0
30	..	0	+	0	++	0	+	0
32	+	..	..	..	..	..	..	..
35	..	+	..	0	+	0	+++	..
36	++	..	..	..	..	..	..	..
42	+++	..	..	..	..	..	..	..
43	..	++	+++	+	+++	0	+++	+
50	..	..	++	+++	+++	0	..	0
56	++++	..	..	..	..	..	+++	..
57	..	+++	..	..	..	0	..	..
65	..	++++	+++	..	..	..	..	..
75	..	..	+++	..	++++	+++	+++	..

* 0 indicates no amyloidosis; + ,early amyloidosis; ++, moderate amyloidosis; +++, moderately advanced amyloidosis; ++++, advanced amyloidosis.

a more satisfactory diet, namely, our so-called stock diet. This diet consisted of 40 per cent ground white bread and 60 per cent powdered whole milk.

Eight groups of twenty-eight albino mice each were formed. The diets fed were as follows:

(*A*) Stock diet of powdered whole milk and ground bread

(*B*) Stock diet plus 0.5 per cent carotene solution[35] (vitamin A)

35. von Euler, B.; von Euler, H., and Hellström, H.: Biochem. Ztschr. **203**: 370, 1928. von Euler, B.; von Euler, H., and Karrer, P.: Helv. Chim. Acta **12**: 278, 1929. Moore, T.: Biochem. J. **23**:807, 1929; **24**:692, 1930; **25**:275, 1931. Collison, D. L.; Hume, E. M.; Smedley-Maclean, I., and Smith, H. H.: ibid. **23**: 634, 1929. Hume, E. M., and Smith, H. H. ibid. **22**:504, 1928. Capper, N. S.: ibid. **24**:980, 1930.

(*C*) Stock diet plus 5 per cent cod liver oil (vitamins A and D)
(*D*) Stock diet plus 1 per cent viosterol (vitamin D)
(*E*) Stock diet plus 5 per cent brewer's powdered yeast (vitamin B complex)
(*F*) Stock diet plus carotene solution plus powdered yeast (vitamins A and B)
(*G*) Stock diet plus powdered yeast plus viosterol (vitamins B and D)
(*H*) Stock diet plus cod liver oil plus powdered yeast (vitamins A, D and B)

The results are given in table 5.

COMMENT

The albino mice on the synthetic diet, without or with one or more vitamins, did not survive a sufficient period to determine the influence of the accessory food substances on the production of amyloidosis. Practically all of them died before 30 to 35 injections were given. The combination of the injections of sodium caseinate suspension and the unsuitable diet made maintenance of life impossible. The control groups (uninoculated) lived from sixty-two to one hundred and eleven days, whereas the groups given injections died within from thirty-one to fifty-four days. Only two groups (one receiving vitamins A and B and one vitamins A, B and C) lived beyond this period, but did not show amyloidosis after 42 and 67 injections, respectively. Furthermore, although the synthetic diets were inadequate under the established conditions, there was no evidence of early amyloidosis and therefore of an acceleration of the onset of this disease.

Of the mice fed the stock diet, most groups showed amyloidosis after from 30 to 35 injections. However, animals the diet of which contained vitamins A and B showed definite retardation of the production of amyloidosis. The group receiving vitamins A and B showed no amyloid after 75 injections; the group fed vitamins A, B and D revealed early amyloidosis after 43 injections, none after 50 injections and again slight evidence after 57 injections. The early changes after 43 injections are explained by the fact that the mice selected for examination appeared ill and in poor physical condition. This illustrates the individual variability in susceptibility to amyloidosis.

Various studies have been made in animals deprived of vitamins A and B, and from our incomplete knowledge of the possible rôle of these vitamins and their effect on tissues we may hypothesize as to the relationship of amyloidosis.

McCarrison [36] noted that lack of vitamin A produced functional and degenerative changes in every tissue of the body, including the spleen, intestines, liver and kidneys.

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36. McCarrison, R.: Brit. M. J. **1**:177, 1919; Indian J. M. Research **7**:167 and 188, 1919.

Wolbach and Howe [37] observed that in man and in the rat, deprivation of vitamin A results in striking epithelial changes. Normal epithelium of the respiratory tract, gastro-intestinal tract, eyes, para-ocular glands and genito-urinary tract are replaced by stratified keratinizing epithelium. Wason [38] and Yudkin and Lambert [39] described such changes in the eyes. Mori [40] noted this change in the mouth, larynx and trachea and the glands.

Findlay and MacKenzie [41] showed that in rats deprived of vitamin A there occur almost complete replacement of the hematopoietic tissue by fibrous tissue and a marked decrease in leukocytic cells.

The importance of vitamin A in the diet for regeneration of blood was reported by Koessler and his associates.[42] Damianovich and his co-workers [43] showed that absence of vitamin A or B in the diet produces a progressive anemia. Abderhalden [44] reported a marked decrease in red blood cells in pigeons deprived of vitamin B. Cramer and his associates [45] stated that lack of vitamin B causes atrophy of the lymphoid tissue throughout the body and lymphopenia in the blood.

Dutcher [46] asserted that vitamin B is a metabolic stimulant. Its absence caused a decrease of the catalase content of the tissues, a depression of tissue oxidation and an accumulation of toxic metabolic products. Bickel [47] contended that absence of vitamin B causes a progressive loss of the power of synthesis and storage of digestive products. Findlay [48] expressed the belief that vitamin B is necessary in the synthesis of nucleic acids. Rohr [49] found that in the absence of vitamin B there occurs a decrease of respiratory activity in the kidney, liver, brain and muscle. Jorstad [50] showed that large additions of vitamin A stimulate the formation and growth and prolong the life of fibroblasts, endothelial cells and other cells that are attracted to the local area

37. Wolbach, S. B., and Howe, P. R.: Arch. Path. **5**:239, 1928.

38. Wason, I. M.: J. A. M. A. **76**:908, 1921.

39. Yudkin, A. M., and Lambert, R. A.: Proc. Soc. Exper. Biol. & Med. **19**: 375, 1922.

40. Mori, S.: Bull. Johns Hopkins Hosp. **33**:357, 1922; Am. J. Hyg. **3**:99, 1923.

41. Findlay, G. M., and MacKenzie, R. D.: J. Path. & Bact. **25**:402, 1922.

42. Koessler, K. K.; Maurer, S., and Loughlin, R.: J. A. M. A. **87**:476, 1926.

43. Damianovich, H.; Branchi, A., and Savazzina, L. A.: Compt. rend. Soc. de Biol. **88**:377, 1923.

44. Abderhalden, E.: Klin. Wchnschr. **1**:160, 1922.

45. Cramer, W.; Drew, A. H., and Mottram, J. C.: Lancet **1**:963, 1921; **2**: 202, 1921.

46. Dutcher, R. A.: J. Biol. Chem. **36**:63 and 551, 1918; Proc. Nat. Acad. Sc. **6**:10, 1920.

47. Bickel, A.: Klin. Wchnschr. **1**:110, 1922.

48. Findlay, G. M.: J. Path. & Bact. **24**:175, 446 and 454, 1921.

49. Rohr, F.: Ztschr. f. Physiol. Chem. **129**:248, 1923.

50. Jorstad, L. H.: J. Exper. Med. **42**:221, 1925.

where coal tar has been injected. According to him, vitamin A protects the cells against the toxic action of the tar and retards their disintegration. Burrows and Jorstad [51] asserted that for growth of body cells a certain substance or substances, believed by them to be identical with vitamin B, were necessary. Furthermore, for extracellular formation in general and for growth and proper structure and function of life, a certain soaplike substance is necessary. This substance is identical with vitamin A. To prevent depletion of this valuable material vitamin A must be supplied.

Summarizing, we may say that deprivation of vitamins A and B leads to functional and structural changes in various organs, including the spleen, liver and kidney, the hematopoietic tissue and the lymphoid tissue. In addition, various metabolic disturbances are produced, such as decreased oxidation, loss of the power of synthesis and storage of digestive products and nucleic acids. Furthermore, there occurs a decreased response to the introduction and elimination of toxic material with early degeneration of the fibroblasts, endothelial cells and other cells. Lastly, inadequate growth and improper general bodily functions result.

These investigations deal with disturbances produced by absence of the vitamins from the diet. It is possible that the supply of these two vitamins in large amounts may cause not only a restoration to optimal normal conditions, but also an enhancement of all of these normal physiologic processes.

In part I a conception of the possible mechanism for the production of amyloidosis was stated. The nature of this condition was considered to be one of a severe and prolonged disorder of endogenous protein metabolism. The abnormal destruction of tissue protein resulted in the production of chemical products which were not readily metabolized to a form which would be utilized or eliminated easily. Failure of disposal of these at a rate commensurate with their formation resulted in their deposition in the tissues.

Carrying this idea further, it may be said that an animal under such conditions is under a strain, and must mobilize all its resources for coping with the amyloid precursor. It is successful in meeting the disturbance and in preventing or retarding amyloidosis to the extent to which it can obtain maximal and optimal function of all the available agents.

An explanation which may account for the retarding influence of vitamins A and B on amyloidosis may be suggested. Vitamins A and B play an important rôle in certain general metabolic processes, and in the satisfactory function and growth of organs (the spleen and liver)

51. Burrows, M. T., and Jorstad, L. H.: Am. J. Physiol. **77**:24, 1926.

and tissues (the hematopoietic and mesenchymal tissue). Amyloidosis is a metabolic disease in which the reticulo-endothelial cells participate. Consequently, an animal will deal more successfully with a disorder when it receives adequate amounts of food factors which are necessary

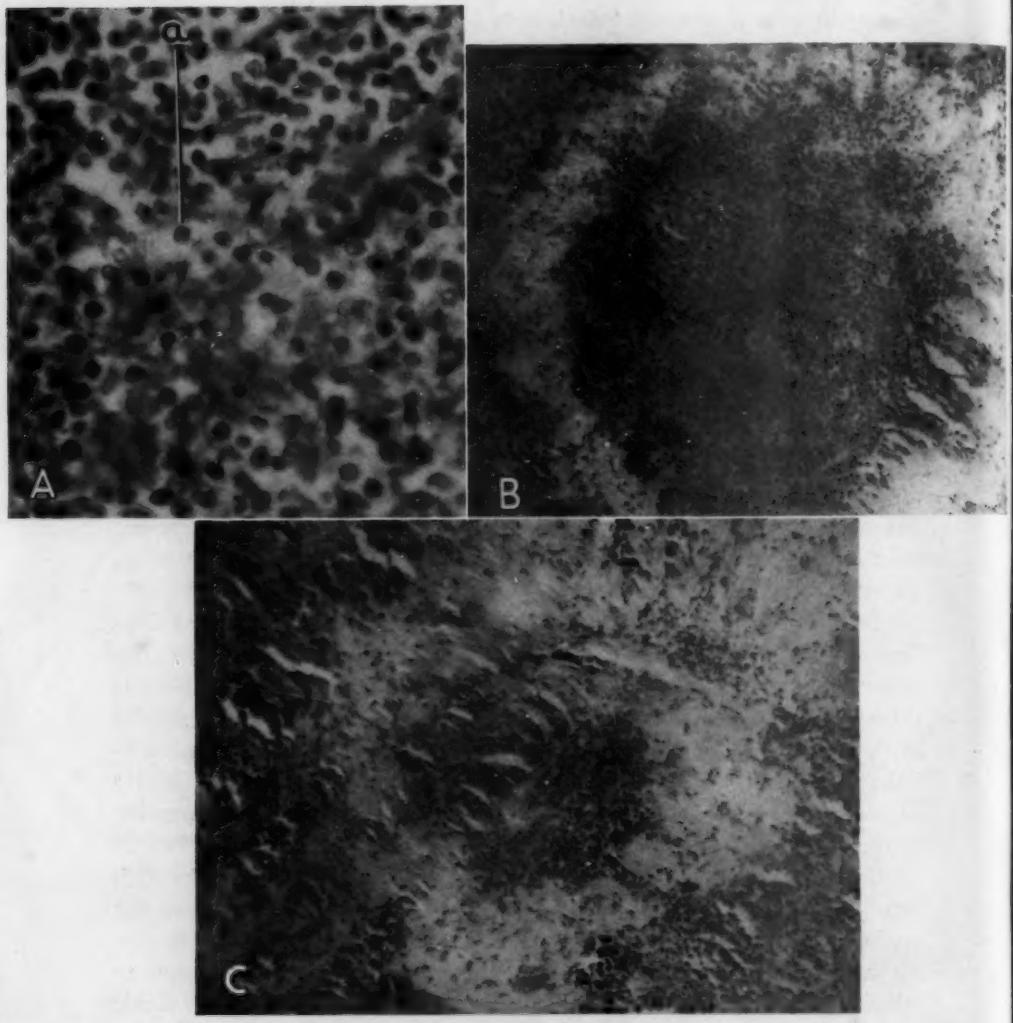

Fig. 2.—Microscopic appearance of the spleen after injections of sodium caseinate. *A*, a higher power photomicrograph of figure 1 *D*, showing the relation of the thickened amyloid fibers (*a*) to the pulp lymphocytic cells; × 400. *B*, the spleen after 48 injections of sodium caseinate, showing a perifollicular ring of moderate amount of homogeneous amyloid. There is no follicular compression or atrophy; × 80. *C*, the spleen after 56 injections of sodium caseinate, showing a vastly increased amount of amyloid, still arranged perifollicularly, with marked compression atrophy of the follicle; × 80.

for satisfactory metabolism and function of tissues concerned in this disturbance.

A minimal number of injections are required, and a certain period of time must elapse before amyloidosis sets in even with inadequate diets or with diets lacking one or all vitamins. It seems, further, that amyloid-like or precursory amyloid changes may be taking place without more than suggestive fixed and wandering cellular morphologic changes in organs being apparent. When, however, an apparent threshold, possibly the resultant of the defense-enhancing factors for the reticular system and the metabolic disturbance, is exceeded, the amyloid suddenly becomes visible in large amounts without going through the progressive phases of its usual morphogenesis. This view seems indicated from the results of this experiment (table 2, group 6). Such a view may further explain the well known individual variability shown by animals.

SUMMARY

Amyloidosis can be produced in all albino mice by subcutaneous or intramuscular injections of a 5 per cent aqueous suspension of sodium caseinate.

The earliest amyloid appears within the fixed and wandering cells of the reticular system. As these cells disintegrate, extracellular amyloid appears, grows in amount and finally replaces the parenchyma of the organ involved.

Amyloidosis is probably the result of an endogenous protein metabolic disturbance. When the rate of formation of these catabolic products exceeds the ability of the tissues to dispose of them, amyloid appears.

With the present technic, amyloidosis cannot be produced in albino rats.

Except in albino mice showing precursory or very early evidences of amyloidosis, no spontaneous resorption of amyloid in definite cases of amyloidosis was observed. Albino mice given a preparation of powdered whole liver in their diet showed resorption only when the degree of amyloidosis was no more than moderate. No retrogression of the disease was noted in advanced cases.

Comparative studies indicate that a well balanced, thoroughly adequate diet exercises a retarding influence on the production of amyloidosis.

The addition of a preparation of desiccated powdered whole liver to the stock diet results in delay of the formation of amyloidosis.

Inadequate or deficient diets do not accelerate the development of amyloidosis.

Mice fed a synthetic and the so-called stock diet to which vitamins A and B were added showed definite evidence of retardation of the production and formation of amyloidosis.

General Review

THE LUNGS AND THE MACROPHAGE SYSTEM

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Reactions to infection depend on the nature of the infectious material (the antigen), the species of the animal infected, the route of infection and also on whether the infection occurred for the first time (normergic or allergic reaction).

The remarkable phenomenon in the process of local reaction to infection is the rapid accumulation in the affected area of cells which previously were present in scant numbers. The nature and origin of this cellular "deluge" has been and remains the bone of contention among investigators.

With the inauguration of the conception of "cellular pathology" the theory of the specificity of cells according to the germinal layer has not been appreciated. For example, in order to explain the occurrence of different varieties of tumors, Virchow devised the theory of metaplasia whereby "one well characterized tissue transforms itself into another equally well characterized but morphologically and functionally different." He disputed that carcinoma originates from epithelial tissues, and up to the end of the century he supported the erroneous observation that Gaucher's disease is a primary epithelioma of the spleen whereby the large "epithelial" cells of which the splenic tumor is made up originate through metaplasia of the connective tissue of the spleen. Pathologists were formerly opposed to Cohnheim's conception of the diapedesis of cells in inflammation, believing that the white blood corpuscles always originate in situ. They likewise disputed the teaching of the "proclivities" of the macrophage, which appeared to them to be rather grotesque.

Cells of which an inflammatory exudate is made up consist of red and white corpuscles, namely, polymorphonuclear leukocytes (granulocytes) and mononuclear cells consisting of lymphocytes, monocytes and macrophages. Whereas the immediate source of the erythrocytes and the granulocytes is commonly accepted as being the circulating blood, the genesis of the large mononuclear phagocytes is debatable.

Metchnikoff, who was the first to stress the rôle of the mesenchyma and to identify the mesodermal cells as the essential elements in the

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field of inflammation, maintained that they originate from the local connective tissue, from the endothelium lining the capillaries and from the circulating blood. Mallory was among the earlier observers who adhered to the conception that the large mononuclear phagocytes always originate from the endothelial cells lining the blood vessels, and accordingly he designated them as endothelial leukocytes. This theory was advocated particularly by Haythorn, Permar, and by Foot, and it dominated the teaching of pathology in this country for more than a quarter of a century. Sabin and her associates, basing their studies on supravital technic, devised the theory of two genetically and functionally different strains of phagocytic cells: the monocyte, which evolves from the reticular cell, and the clasmatocyte, which is of endothelial origin. Aschoff traced the origin of the macrophages to the local connective tissue (histiocytes, or tissue cells), while Maximow produced evidence to show that the polyblast or macrophage in inflammation has a double origin: hematogenous and histogenous. In the common loose connective tissue and in the serous membranes the resting and wandering cells are: adventitial cells, the pericytes and the clasmatocytes. Maximow included in the group of hematogenous cells the monocytes of the blood and also the lymphocytes, which according to his experiments, transform themselves into macrophages. This concept was originally expressed by Metchnikoff, who wrote: "Si les lymphocytes mêmes ne sont encore point des phagocytes, ils le deviennent bientôt après s'être transformé en cellules epithélioides." ("If the lymphocytes are not yet phagocytes, they soon become so after having been transformed into epithelioid cells." Lewis and Lewis concluded from their studies on tissue cultures that the mononuclear monocytes, the macrophages and the epithelioid cells are related to temporary functional variations. These authorities could find no definite evidence of the transformation of the lymphocyte into a monocyte. They assumed that the epithelioid cells of tuberculous lesions are derived either directly or indirectly from the mononuclears of the blood. Von Möllendorf, in recent publications, claimed that the fibroblast, too, is capable under proper circumstances of transforming itself into a macrophage, but this was energetically contradicted by Maximow in articles which were published shortly before his death.

Before presenting the discussion on the mesenchymal reactions of the lungs, it may be well to review briefly the opinions regarding the normal histology of the air sacs.

STRUCTURE OF THE PULMONARY PARENCHYMA

The structure of the respiratory portion of the lungs was investigated by numerous authorities, particularly by Miller, who summarized his views in the Harvey Society Lectures of 1924 and 1925. Whether the

air sacs are directly connected with the terminal bronchioles or whether there exists between them an interposed cavity, an atrium, as was constructed by Miller, is irrelevant to the present report.

The alveolar wall was described by von Kölliker and Schaffer as being a homogeneous membrane containing no collagenous fibers but endowed with elastic fibers and harboring capillaries. Von Möllendorff and Russakoff described the septums as being composed of a fine network of reticulum fibers which rarely insinuate themselves between the capillaries and the lining epithelium. The reticulum fibers were looked on by Orsós as being collagenic. The same observer discriminated between two varieties of elastic fibers: respiratory, which are made up of coarse fibers not related to the capillaries, and intercapillary, the fine fibers of which are interwoven with the capillaries. A few histologists found a basal membrane covering the septums, the supporting substance of which contained a sparse number of mesenchymal cells.

Of particular interest is the question relating to the cells lining the air sacs, the so-called alveolar epithelium. Von Kölliker, whose opinion was shared by most histologists, found that the air sacs are lined by large non-nucleated plates interspersed with groups of small nucleated epithelial cells. "With the general acceptance of Kölliker's description of the alveolar epithelium," wrote Miller, "all investigation of the alveolar epithelium practically ends, and his illustrations are frequently reproduced." In a recent review Miller stated that "in the normal lung, the epithelium lining the alveolar wall is made up of thin, flattened, nucleated squamae which are closely applied to the alveolar wall, and it is a continuous epithelium."

Bloom, in Maximow's textbook of histology, stated that "the details of the structure of the respiratory portion of the lung have been only partially elucidated." "There can be little doubt," he stated further, "that the so-called 'nucleated alveolar epithelial cells' of the older authors are composed of certain pericapillary cells—probably also the endothelial cells of the capillaries and even the red blood corpuscles within these vessels."

The problem, which superficially appears to be that of cytology, is in reality one of the physiology and pathology of the lungs. This will be elaborated in the following discussion.[1]

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1. "The problem of general physiology," wrote Claude Bernard, "is centered around the histologic elements. The physiologist will understand the mechanism of life and will be in a position to influence it scientifically when the circumstances which influence the cells in their intimate organic medium have been established." (Rapport sur le progrès de la physiologie générale, Paris, J. B. Baillière et fils, 1863.)

THE DEFENSE RÔLE OF THE LUNGS

Observers in the past expressed the hypothesis that the function of the lungs is probably not limited to gaseous exchange. A study made by Fort in 1867 was to the effect that these organs display characteristics of secretory organs and accordingly he used the term pulmonary gland, which was later accepted by Roger and by Aschoff. The function of the lungs is probably complex. From the point of view of circulation they are the point of convergence of the body fluids, and material which happens to invade the circulation invariably reaches the lungs. Then, they are the single visceral organ which is in direct contact with the outside world. They are, therefore, liable to receive dust, bacteria and other matters directly from the air. It is remarkable that in spite of the exposed position the pulmonary parenchyma usually contains no bacteria. Moreover, investigations have shown that when pathogenic microorganisms are experimentally introduced into the respiratory portion of the lungs by way of the trachea, they rapidly disappear, in most instances without causing disease.

What is the mechanism whereby the lungs maintain their sterility in normal conditions? How do they react in instances of inflammation?

The defensive rôle of the organism in general is attributed to the connective (mesenchymal) tissues and particularly to a variety of cells which are derived from the embryonic connective tissue, the mesenchyma. These cells, to which reference has previously been made, which are known as macrophages, clasmatocytes, histiocytes and polyblasts (also adventitial cells and pericytes), were originally described by Metchnikoff, who produced evidence that they are concerned with the defense of the organism by ingesting bacteria (phagocytosis) which have made their way into the body and by producing antibodies and forming antitoxins. Attention has been called particularly to the macrophage since the introduction in experimental pathology of supravital staining of animals. Goldmann, after injecting solutions of pyrrol blue into the veins of animals, noted that the dye was taken up by one variety of cells in a special manner, being deposited in the cellular cytoplasm as fine and coarse granules. The red blood cells and the elements of lymphoid and myelogenous origin remained unstained. He observed that these cells are found in all parts of the body, and he identified them with the macrophage of Metchnikoff, the functions of which are those of defense and metabolism. More recent investigations are to the effect that the same variety of cells is also concerned with the defense against and the formation of neoplastic conditions. In brief, at present the mesodermal tissue is looked on as an "organ" performing multiple functions. It is of interest to know whether the lungs, too, are endowed with such an "organ."

In order to investigate the problem I conducted experiments which can be divided into two groups: (1) "sterile" infections (also defined as "model" infections), consisting in the administration to animals of vital dyes and oils respectively, and (2) virtual infections of animals with pathogenic micro-organisms.

I. "MODEL" INFECTIONS

Vital Staining.—Metchnikoff was the first to use dyes in order to observe the behavior of the large mononuclear phagocytes. By placing living sponges in fluid containing carmine or indigo respectively, he noticed that the dyes were taken up by the large mesodermal cells, the macrophages.[2] Bouffard and Ribbert were also using the method of "dyeing" animals in vivo in order to study the reaction of tissues. However, they did not fully appreciate the importance of this technical procedure, and their conclusions were of limited value. Goldmann, whom I have quoted, was the first to emphasize and broaden Metchnikoff's conception that the cells which take up the dye represent a network spoken of as a system or an apparatus spread over the body and possessing common physiologic traits of which the affinity for vital dyes is of particular interest because of the fact that this "earmark" makes them readily identifiable. Thus, by using the method of intravital staining, one is able to recognize the cells of the middle embryonic layer. Aschoff designated this group of cells as the reticulo-endothelial system. I use the term macrophage system, which is rather expressive, which does not prejudge the alleged origin of the cells (reticular and endothelial) and, finally, which renders homage to Metchnikoff, who first observed them.

In examining the tissues of the vitally stained animals, it was observed that while the repeatedly injected dye had stained the abdominal, pelvic and the reproductive organs deeply, it had stained the lungs to a slight degree only. Under the microscope the cells of the histiocytic group were abundantly stained in the bone marrow, the liver, the spleen and the lymph nodes. In the lungs the dye was characteristically stored in the adventitial cells or the pericytes of the larger vessels and in the interalveolar septums. The other pulmonary structures remained either unstained or only faintly tinged with blue.

From similar observations previous investigators concluded that the lungs contain sparse numbers of macrophages.

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2. Metchnikoff was likewise the first to apply the method known as blockade of the macrophage (reticulo-endothelial) system. He noted that under the conditions of his experiments guinea-pigs tolerated intraperitoneal injections of trisulphide of arsenic, the toxic substance being rapidly phagocytosed by the macrophages. But when the injection of arsenic was preceded by that of a solution of carmine the guinea-pigs succumbed. He observed that the large phagocytes "gorged" with the dye remained indifferent to the arsenic which caused the death of the animals.

But when the dye was injected into the lungs via the trachea, the cells lining the air vesicle revealed the following changes: They had enlarged, and their cytoplasm, which had become abundant and "foamy," often contained droplets of pyrrol blue, which was distributed in the manner of histiocytes. Then a number of them seemed to abandon their customary seat on the septum to become wandering alveolar phagocytes, forming the intra-alveolar (inflammatory) exudate.

The endothelium of the capillaries did not show any cytopoietic properties, and the participation of the monocytes from the blood in the process occurred at a later date, probably when the dye had become diffused through the organism in a concentration sufficient to irritate the mesenchyma, leading to proliferation and "release" of local phagocytes.

The origin of the free phagocytic cells found in the air sacs ("dust cells," "heart disease cells") and of the epithelioid cells was the object of investigation by the earliest pathologists. Whereas the partisans of Cohnheim's theory maintained that these cells have reached the lungs from the blood stream by diapedesis (Slavjanski, von Ins), the observers who were opposed to Cohnheim's theory traced the cells to those lining the walls of the alveoli (Ruppert, Arnold) and to those lining the alveolar and bronchial walls. Among modern investigators, Briscoe, Seemann and Westhues, from Aschoff's laboratory, Sewell and Rosin have adopted the views of Ruppert and of Arnold. Permar, a partisan of Mallory's conception, in his studies with vital dyes and also in experimental pneumonia found that "they [the monouclear phagocytes] arise, in part at least, if not predominantly, from the fixed endothelial cells of the capillary and subcapillary vessels, in the walls of the atria, sacculi alveolares and alveoli pulmonis." Foot, too, published numerous articles in defense of this conception which he has recently discarded for the hematogenous theory. The results of Olch and Ballon's studies on dogs coincided with those described by me. Huguenin and Delarue, who also used dogs in their experiments, have corroborated my observations on small laboratory animals.

Intratracheal Injection of Oils.—When the lungs were removed and examined about one hour after the intratracheal injection of oils (iodized poppy seed oil 40 per cent, cod liver oil and liquid purified petroleum benzine), the reaction they produced was essentially that of the cells lining the wall of the air sacs. These cells revealed a cytoplasm which contained vacuoles of various size and a hyperchromatic round or oval nucleus (fig. 1). They protruded above the surface of the alveolar wall in a budlike manner, and a number of them lay free in the alveoli and contained phagocytosed fat in their cytoplasm. Within from twelve to twenty-four hours following the injection, the cells of the alveolar wall showed a marked increase in number and also in size; they appeared as large foam cells, thus leading to thickening of the alveolar septums. At the end of the second and third days the cellular proliferation caused atelectatic areas. In places the greatly enlarged cells lining the septum and those accumulated in the pulmonary alveoli produced continuous cellular masses resembling xanthomatous nodules or agglomerations of proliferated cells like those seen in Gaucher's disease (fig. 2).

Neither the endothelium of the capillaries nor the bronchial epithelium participated in the reaction. The afflux of phagocytic cells from the blood stream was insignificant, and their rôle in the formation of the intra-alveolar exudate was slight.

Waldeyer was probably the first to note that "the embryonic cells of the connective tissue," or *Plasmazellen*, take up fat avidly; he spoke

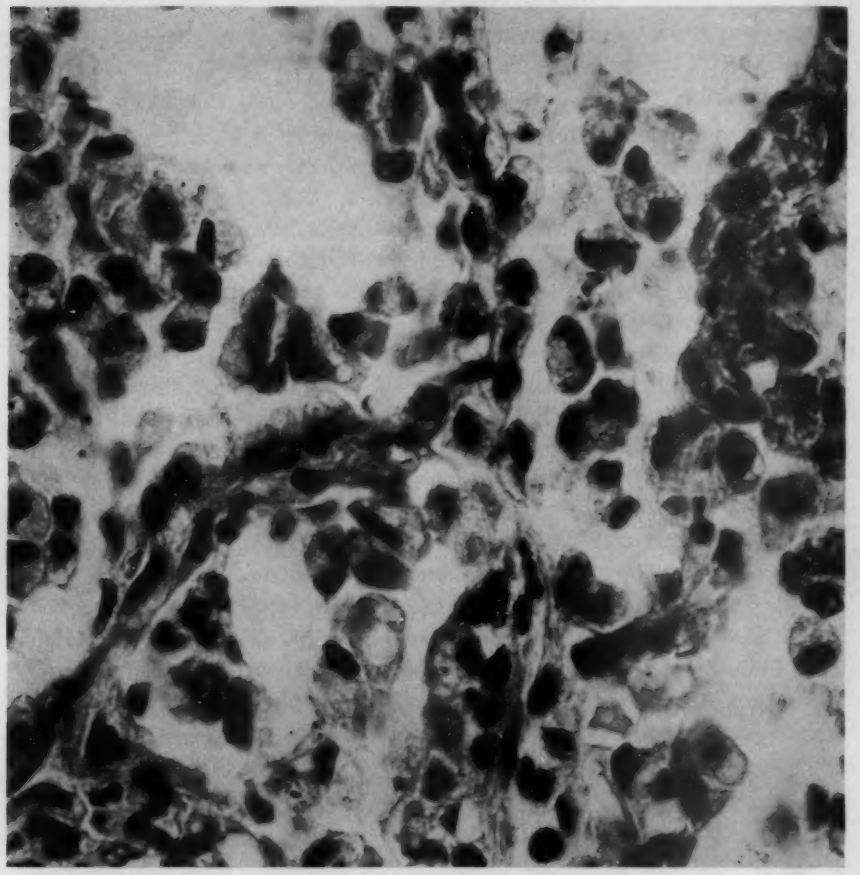

Fig. 1.—Fixed and wandering cells of the air sac, showing their similarity and their close resemblance to macrophages (histiocytes).

of these cells as "grosse rund Zellen welche besonders gern Fett aufnehmen" ("large round cells which absorb fat with avidity"). François noticed that these cells "transform" themselves into fat cells which at one time was considered to be a pathologic condition, but which subsequent observations have revealed to be a physiologic metabolic change. Schultze observed the rôle of these cells in diabetic lipemia. In per-

forming a necropsy on a patient with diabetes and lipemia, he found an enlarged spleen which on histologic examination showed proliferated and hypertrophied reticular cells (fig. 3). He expressed the opinion that the spleen most likely plays a rôle in the metabolism of lipoids and suggested that this property resides in the reticular cells (splenic macrophages, or splenocytes). This was subsequently corroborated by

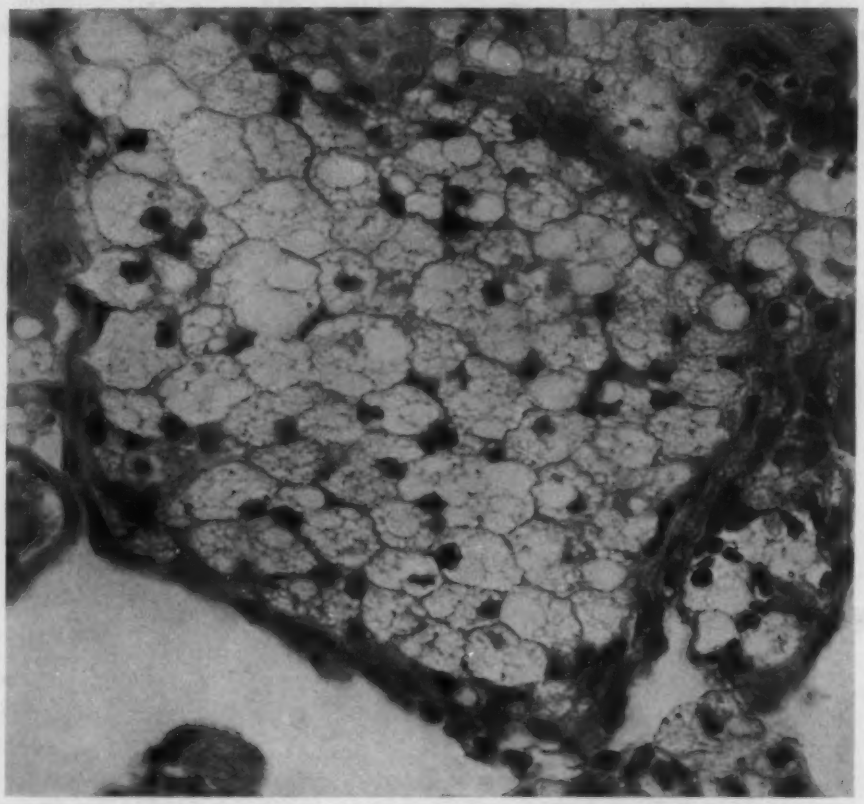

Fig. 2.—An intra-alveolar xanthomatous nodule made up of proliferated cells of the alveolar wall.

many observers, who also found that similar processes occur in nephrosis, in obstructive jaundice and in other pathologic conditions.

More recent studies on Gaucher's and on Niemann-Pick's disease and also on the disease known as the Schüller-Christian syndrome (xanthomatosis) have likewise revealed that the lipoids whose metabolism is disturbed are phagocytosed essentially by the macrophages (Rowland). Similarly, experimental studies by Anitchkow and by Zinserling revealed

that small laboratory animals which were fed an excess of cholesterol emulsified in sunflower oil showed in the intima of the vessels large cells studded with lipoids, which were readily identified as macrophages. Thus, when rabbits are fed an excess of pure cholesterol or yolk of egg, the lipoids are "stored" in the spleen and the bone marrow, which, on microscopic examination, reveal that the material is deposited exclusively in the reticular cells and in the cells which line the sinuses of the spleen. As the result of the excessive feeding of cholesterol these

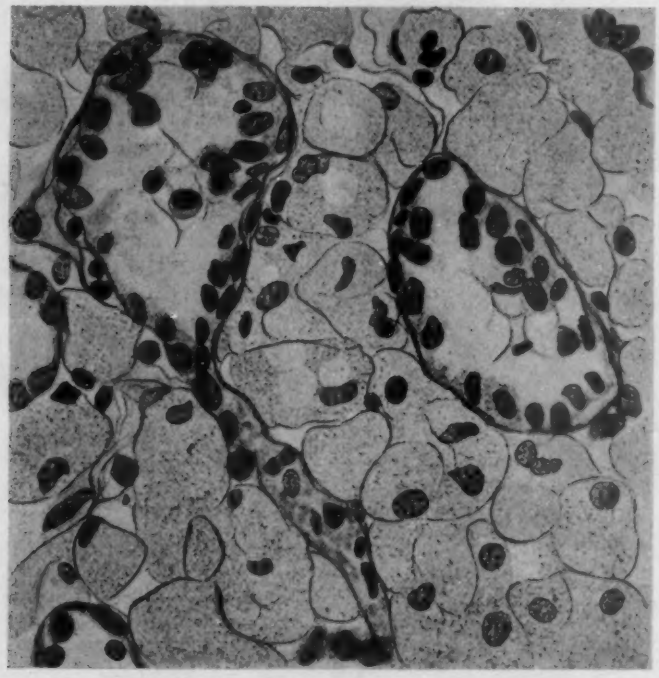

Fig. 3.—Structure of the spleen in diabetic lipemia (Schultze). The dilated sinuses lined with proliferated and hypertrophic macrophages were formerly described by investigators as "cystic structures lined with epithelial cells."

cells enlarge and proliferate, forming wide agglomerations in the splenic pulp and also in the sinuses. In the bone marrow, the orally introduced fatty substances lead to cellular changes similar to those found in the spleen. Anitchkow noticed that cholesterol artificially increased in the organism is deposited largely as anisotropic fat in the macrophages of the connective tissue, with the formation of typical xanthoma cells.

Oils introduced into the respiratory portion of the lungs via the trachea cause a reaction in which the alveolar "epithelial" cells (also the

peribronchial and perivascular macrophages) are primarily involved. The behavior of these cells is a counterpart of that of the macrophages in the spleen, liver and bone marrow. Seemann, after injecting fine emulsions of cholesterol into the circulation of laboratory animals, observed that in the lungs the lipoid substance was "fixed" by the cells lining the alveoli, being deposited and "stored" in their cytoplasm, as is done by the macrophages elsewhere in the body.

These observations are of interest in connection with the hypothesis expressed by numerous writers that the lungs play a rôle in the metabolism of fat. Roger compared the pulmonary action on fatty substances with the action of the liver on carbohydrates. This comparison was suggested by the fact that absorbed albumins and carbohydrates pass directly into the liver, while fatty substances are transported by way of the lymphatics and the thoracic duct to the right side of the heart and to the lungs. Extensive studies by Derman and Leites were to the same effect. Markowitz and Mann, in a more recent report, stated that the results of their experiments "have not furnished evidence for the supposition that the lung plays an especial rôle in the metabolism of fat." However, they, too, found that in one experiment, in which a highly emulsified preparation of fat was injected intravenously, there was a huge increase of fat in the lung and a definite diminution of fat in the blood from the femoral artery as compared with blood removed from the right ventricle.

Apparently the problem is complex, and further experiments will be required. As Ranvier expressed it: "Quoi de plus aisé que de montrer aujourd'hui la présence de glycogène dans le foie. Cependant, Claude Bernard, qui était un homme de génie, n'y est arrivé qu'après bien des années de recherches et par une série de tâtonnement." ("Nothing is easier today than to demonstrate the presence of glycogen in the liver; yet Claude Bernard who was a genius, succeeded in doing so only after numerous attempts and years of research.") [3]

II. VIRTUAL INFECTIONS

Infection of Animals with the Anthrax Bacillus Via the Trachea.—The "model infections" have shown that the lungs possess a cellular "apparatus" which to all appearances is a counterpart of the macrophage apparatus found throughout the body. It also became apparent that the cells of which it is made up are chiefly those which line the air sacs and which are commonly designated as alveolar epithelium. The

3. I have observed in man and in lower animals that the alveolar epithelium plays a rôle in the local (extrahepatic) formation of bile. By inducing hematomas in the lungs of animals, bilirubinemia can be demonstrated.

experiments were undertaken in order to study the behavior of these cells in instances of virtual infection with pathogenic micro-organisms.

The experiments with the anthrax bacillus can be divided into two series: (1) a series in which the rabbits receiving the injection of the gram-positive bacillus by way of the trachea were left alive for observation, and (2) a series in which the animals were killed at intervals of from five minutes to three weeks after the intratracheal infection. The experiments showed that manifold fatal doses of the anthrax bacillus can be introduced into the lungs of rabbits with impunity when the micro-organism is brought directly in contact with the pulmonary tissue, provided that the extrapulmonary tissues are spared contamination. The micro-organisms so inoculated are retained by the lungs, where they are destroyed within a few hours after injection by phagocytosis, in which the cells found in groups along the wall of the air sacs (alveolar epithelium) play the outstanding rôle.

Although the anthrax bacillus belongs to the micro-organisms that have been discovered earlier (Davaine, Pasteur), the mechanism of death caused by this *batonnet* remains to be explained. As no observer has succeeded in isolating a toxin from this micro-organism, it was presumed that it causes death not by endotoxins or exotoxins but by virtue of its tremendous proliferation within the circulation, which results in occlusion of vessels culminating in asphyxia. However, as has been stated, recent investigations have revealed that when the bacillus is injected into the blood of animals it disappears rapidly from the circulation; it is lodged in the internal organs and reappears in the peripheral circulation shortly before the animals' demise (Besredka). Septicemia is, then, a terminal stage, simply heralding the fact that the host has lost the fight and that the offender is released. It is also remarkable that in this infection, probably more than in any other, local trauma plays an outstanding rôle in the spread of the disease. Finally, it was noted by observers both formerly and recently that the micro-organism is harmless to laboratory animals when injected into the lung with the precautions used in my experiments (Morse, Hildebrandt, Grammatchikoff, Snel, Besredka, Balteano, Brocq-Rousseau and Urbain, Combiesco, Gracia).

Besredka was the first to stress the fact that in the lower animal the skin is the sole organ that is susceptible to the anthrax bacillus, whereas other organs or structures resist infection. Thus, he stated that "chez le cobaye il n'existe qu'un organ pour lequel la bactéridie ressent une réelle affinité, organ dans lequel elle peut s'implanter, au sein duquel elle peut croître et se multiplier: cet organ est la peau." (In the guinea-pig only one organ exists for which the anthrax bacillus shows a virtual affinity and in which it is able to implant itself, grow and multiply; that organ is the skin.") "La bactéridie," he stated further, "que pénètre ailleurs que dans la peau ou le tissu sous-cutané,

passe évidemment inaperçue de l'animal: aussitôt arrivée dans le péritoine ou dans le poumon elle y est aussitôt phagocytée et digérée." ("The anthrax bacillus which enters by other means than the skin or the subcutaneous tissue remains unnoticed by the animal; in the peritoneum or in the lungs it is phagocytosed and digested the moment it has invaded these organs.")

The experiments with the anthrax bacillus belong to the series of postwar studies which served Besredka as a point of departure for his conception of local immunity. His work on local immunity to pathogenic micro-organisms has been corroborated by a number of observers, who also were concerned with the mechanism of this phenomenon. For details on this phase of the problem the studies by Gay and his associates and by Cannon and Pacheco are of interest. Gay, who made extensive studies on the subject, produced evidence that "the clasmatocytes or 'tissue macrophages' are in part, if not entirely, responsible for the natural resistance of rabbits to experimental streptococcus infection." He also made the important observation that the "native" resistance varies from structure to structure, depending on the abundance of macrophages with which the region is provided. Thus, the peritoneal cavity is at least a thousand times more resistant to streptococcic infection than the pleural cavity. It is also remarkable that the macrophages are mobilized from the adjacent connective tissue and not from the circulating blood. Cannon and Pacheco found that the local immunity of the skin resulting from previous immunization by intracutaneous injections of staphylococcus vaccine is predominantly cellular in type, with the tissue macrophages playing the dominant rôle, owing to increased numbers and also, probably to increased metabolic activity.

From the foregoing observations it is apparent that the macrophage is the element *par excellence* which is concerned in the process of local immunity of tissues to invaders. "Les propriétés humorales," wrote Metchnikoff, "ne représentént qu'une certain fraction dans l'ensemble des phénomènes de l'immunité, cette dernière étant dominée par des propriétés cellulaires, qui apparaissent dans la presque totalité de cas d'immunité naturelle ou acquise." ("In the complete phenomenon of immunity, the humoral properties represent but a fraction, the phenomenon being dominated by the properties of the cells which are conspicuous in nearly all cases of natural and acquired immunity.") This statement, made more than thirty years ago, has been recently confirmed by observers who used modern methods of investigation, such as tissue cultures.

Apparently, in the experiments with the intratracheal injection of the anthrax bacillus the same defensive mechanism intervened. It is, however, remarkable that the outstanding rôle of the scavenger which

disposes of the pathogenic *batonnet* was played by the cells lining the wall of the air sacs, which from time immemorial have been regarded not as macrophages but as epithelial cells. This observation was particularly evident from the infection of animals with the tubercle bacillus which is described in the following section.

Infection of Rabbits with Tubercle Bacilli by Way of the Trachea.—In initiating a study on experimental tuberculosis, the respiratory route of infection was chosen in order to imitate the technic of the "model" infections with dyes and oils. A survey of the literature has revealed a few incomplete reports on this method of infection with Koch's bacillus. Indeed, the results reported by previous investigators were usually based on hematogenous methods of infection whereby a condition of acute sepsis with Koch's bacillus was induced in animals. It is accepted that in man tuberculosis is as a rule initiated by inhalation of the acid-fast bacilli, whereas a primary infection by way of the blood stream is very rare. In the lower animals the two forms of infection respectively cause diseases the natural histories of which vary to a great extent, the aerogenous being probably closer to the form observed in man. The experiments were conducted on full-grown rabbits.

Microscopic examination of tissues revealed that when the tubercle bacilli reached the air sacs the reaction was instantaneous: within a few minutes there occurred proliferation, as well as morphologic changes of cells lying in and on the septums. The latter cells which are normally barely visible, giving to the air sac a quasi-naked appearance, revealed characteristics that are proper to fixed or wandering macrophages (monocytes, clasmatocytes). A number of these cells lay free in the alveolar lumen, showing phagocytosis of the tubercle bacilli. Tubercle bacilli were also found in the cytoplasm of some of the enlarged sessile cells. Within the first hour after the infection primitive tubercles made up of the cells lining the air sacs (respiratory epithelial transformed into epithelioid cells) were seen scattered over the sections. At a later period outlines of giant cells could be made out, resulting from agglutination of individual cells and also from the nuclear division of cells.

As a rule, newly formed cells proliferated inside the lumen of the alveolus, (fig. 4) forming a microscopic pneumonic focus or a "parenchymatous alveolitis" (*alvéolite macrophagique* of the French writers). This process ordinarily involved a group of several alveoli, the septums of which could be outlined early in the disease.

In addition to the prompt morphologic changes and the rapid proliferation, the sessile and free cells displayed instantaneous phagocytosis of the acid-fast bacilli. Not only did the morbid process appear to advance by proliferation of its own elements, but at the borders it "converted" the adjacent cells. Here, too, the cells, while still resting on the septums, had taken on new morphologic aspects closely resembling those of the large monocytes. In proximity to the primitive tubercle these cells could be seen to "stream" singly or in groups to join and enforce the already formed miliary nodule. Thus, when the agglomerated tubercle had reached considerable dimensions and its center had begun to degenerate

(caseation), its periphery was made up of new elements that resulted not only from proliferation of its own cells but also from the mobilization of those resting in the adjacent area.

The endothelium of the capillaries of the septums showed no particular changes. The lumens of the septal capillaries contained a moderate number of red cells. The pulmonary lymphocytes did not seem to play a rôle in the provision of the first "waves" of macrophages.

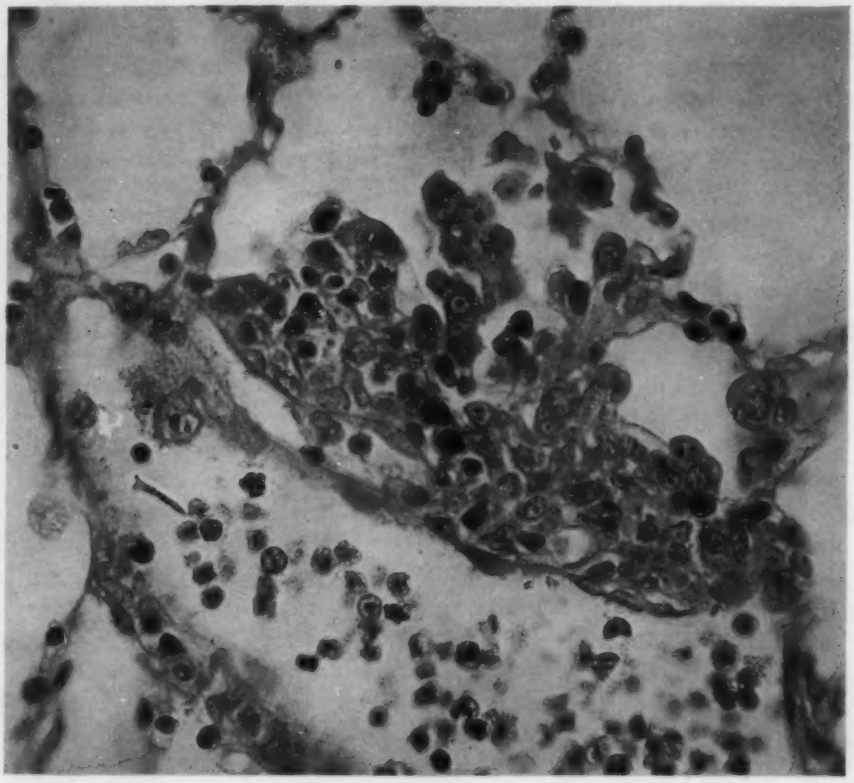

Fig. 4.—Section of the lung of a rabbit infected with tubercle bacilli, showing the early stage of parenchymatous alveolitis.

Virchow regarded the tubercle in tuberculosis as a heterologous growth (neoplasia), taking its origin from connective tissue analogous to that found in neoplastic diseases. However, the opinion was prevalent among his contemporaries that the miliary tubercle is formed from all kinds of fixed tissues: the epithelium of the bronchi and the respiratory tract, the endothelium lining the blood vessels, the polygonal hepatic cells and the epithelium lining the bile ducts, renal epithelium, muscle fibers and the like (Thaon, Grancher, Strauss, Baum-

garten and others). As has already been stated, although the cell dominated the teaching of pathology, cellular specificity was but vaguely understood until the advent of Metchnikoff.

According to Metchnikoff, "the tubercle is made up of a collection of phagocytes of mesoblastic origin, which flock toward the areas where the bacilli are located and engulf them." "In the lungs," he stated elsewhere, "the tubercle is formed at the expense of the endothelial cells of the blood vessels with assistance of leukocytes." Borrel, who, after Metchnikoff, made a comprehensive experimental study on tuberculosis, wrote as follows: "Le système lymphatique est la gangue ou se developpent les tubercules, et non le tissue conjonctif. La cellule tuberculeuse est toujours une cellule lymphatique." ("The lymphatic system and not the connective tissue is the soil where the tubercles develop. The tuberculous cell is always a lymphatic cell.") Calmette fully subscribed to Borrel's findings by stating that "dans les poumons comme dans les sereuses, l'élection des tubercules autour des vaisseaux est due a cette particularité qu'ils se developpent presque exclusivement dans le système lymphatique. Ce dernier est la gangue ou se constituent les tubercules, et non le tissue conjonctif comme le prétendait Virchow." ("In the lungs as in the serous membrane the elective seat of the tubercles around the vessels is due to the fact that they develop almost exclusively in the lymphatic system. The latter is the terrain where the tubercles are formed, and not the connective tissue, as was claimed by Virchow.") Baldwin, Petroff and Gardner, in a recent monograph, emphasized that "without exception tubercle is formed in the depth of the tissues, by preference in lymphoid masses, but where the latter do not occur, in and about lymphatic vessels; never does the tubercle start upon an epithelial surface. Applying these propositions to the case of the lung, tubercle could not develop upon the surface of the respiratory epithelium, nor would it be expected within the wall of the air spaces which are devoid of lymphatic vessels." Finally, Krause stated that "added years of uninterrupted observation of the primitive tubercle have served only to strengthen the idea of at least one worker, that this parent cell exists *in situ*, and in a thoroughly topographical sense, may be thought of and designated as a fixed tissue cell; that it is not exuded from the blood or lymph, or called as by *taxi*, from a distance in response to tubercle bacilli."

Pathologists today share fully Metchnikoff's views as to the mesodermal origin of the epithelioid cells of which the tubercle of the lung is made up. They are, however, in disaccord as to their source. As in the case of the origin of inflammatory exudate elsewhere in the body, investigators have adhered either to the theory of endothelial leukocytes of Mallory, or to that of the hematogenous origin of the epi-

thelioid cells; to the view that they arise in local connective tissue or to the view that they have a double origin—hematogenous and local.

The endothelial origin of the pulmonary epithelioid cells have been combated by Aschoff and by Maximow. I, too, could find no evidence of the participation of the endothelium of the pulmonary capillaries in the provision of the epithelioid cells. This conception has been recently abandoned by the "younger" pathologists (Foot, Gardner) and apparently by Mallory himself. Thus, in a study made in Mallory's laboratory, Nye and Parker stated as follows: "In the past, many writers have claimed that the capillary endothelium in the lungs possesses great phagocytic powers, but few at present hold this view. There is no evidence that the monocytes in the lungs are derived from the *circulation* or from the *capillary endothelium* [italics mine]." They believed that the source of the pulmonary phagocyte lies in the lungs themselves and not in the extrapulmonary tissues, but they were not certain as to its precise location. Gardner and Smith, too, found that migration (into the lungs) of cells from the blood stream is eliminated because alveolar phagocytes accumulate after the death of the animal, when the blood is no longer circulating.

Apparently Foot is the sole author who recently expressed the opinion that the alveolar phagocytes are of hematogenous origin. In view of the fact that he attributed the same conception to Metchnikoff, it is pertinent to look into this investigator's statements.

In his first article on phagocytosis of Koch's bacillus, published in 1888, Metchnikoff wrote: "Die Lungen-Tuberkelbildung bei Kaninchen (nach Injektion der Kulturen in die Ohrvene) erfolgt in *wenigen Tagen* wobei man starke Anhäufungen von *fertigen* Epithelioidzellen, sowie von Lymphozyten beobachten kann [italics mine]." ("The formation of tubercles in the lungs of rabbits [after the injection of cultures in the vein of the ear] follows within a *few days,* whereby one observes massive accumulations of *fully developed* epithelioid cells and lymphocytes.") The source of the "*fertige Epithelioidzellen*" in the lungs was not specified. In his "Leçons sur la pathologie comparée de l'inflammation" he stated: "Après les leucocytes, ce sont les vaisseaux et leurs endothéliums qui jouent le rôle le plus important dans l'inflammation . . . . les éléments endothéliaux ont conservé encore plusieurs traits de mobilité, attestant leur origin. . . . Le tubercule pulmonaire se forme aux dépens des cellules endothéliales des vaisseaux sanguins avec le concours des leucocytes." ("After the leukocytes, the vessels and the endothelium play the most important rôle in inflammation . . . the endothelial elements in accord with their origin have preserved many features of motility. . . . The pulmonary tubercle is formed at the expense of the endothelial cells of the blood vessels

with the assistance of leukocytes.") The source of the latter was described by him in his monograph "L'immunité dans les maladies infectieuses," in which he wrote: "For a long time the large 'dust cells' of the respiratory channels were looked on as being epithelial cells that were capable of taking up carbon particles, micro-organisms and other foreign bodies. In reality these elements are none other than white corpuscles that have immigrated into the alveoli and bronchi." In the spleen and the lymph nodes the tubercle, according to Metchnikoff, results from a collection of the large mononuclear phagocytes of these organs and not from outside sources.

In connection with Metchnikoff's opinion, it is of interest to cite that of his contemporary, Borrel, on the same subject. Borrel, who used the hematogenous method of infection with the tubercle bacilli, found, as has already been stated, that the cells in the tuberculous lesion are always of "lymphatic" origin. In another section of his article dealing with the alveolar response he wrote as follows: "Dans les alvéoles il existe une catégorie de cellules qui jouent le même rôle que les grands leucocytes mononucléaires dans les vaisseaux, ce sont les cellules à poussidères, les *Staubzellen* des auteurs allemands." ("There exists in the alveoli a category of cells which play a rôle equivalent to that of the large mononuclear leukocytes found in the blood vessels; these are the dust cells, the *Staubzellen* of the German authors.") He did not observe them in the blood-borne infection with Koch's bacillus, but he mentioned casually that, as a result of the "inhalations de grandes quantités de bacilles tuberculeux, on constate alors au microscope, des premiers jours, l'envahissement des alvéoles pulmonaires per une énorme quantité de cellules à poussières; la plupart de ces cellules contiennent des bacilles qui sont comme noyés dans un épanchement non moins considérable de leucocytes polynucléaires." ("After the inhalation of large quantities of tubercle bacilli, microscopic observation shows from the first the invasion of the pulmonary alveoli by an enormous quantity of dust cells. Most of these cells contain bacilli, which appear as if drowned in a not less considerable exudate of polymorphonuclear leukocytes.") Apparently Borrel discriminated between two strains of cells: the immigrated large mononuclear leukocyte and the "dust cell." He suspected that the latter was indigenous in the lung and that it displayed its activity in massive aerogenous infection.

It may be seen that the theory of the essentially hematogenous origin of the dust cells and of the pulmonary epithelioid cells forming the tubercle was erroneously attributed to Metchnikoff.

Of particular interest in this respect are the experiments of Lang and of Timofejevski and of Benevolenskja, who made cultures of lung tissue inoculated with tubercle bacilli. In experiments, in which circu-

lation was excluded, these investigators showed that the lungs and no other organs represented the source for the alveolar phagocytes.

The findings of Borrel, of Calmette and of Baldwin and his associates that "the tubercle is formed . . . by preference in lymphoid masses" resulted probably from observation on hematogenous infection with the tubercle bacilli. The vascular supply of the lymphoid tissue found within the lung of the rabbit is derived from the pulmonary artery (Miller). "Therefore," wrote Krause, "in the rabbit, bacteria that have gained the general venous system will, in part at least, be conveyed to the lymphoid tissue directly." However, even here, the tubercle, i. e., the epithelioid cells, originate not from the lymphocytes, but from the clear central zone of the reticular cells, which are none other than macrophages.

Thus the notion held by authorities that the blood stream and the endothelium are the essential contributors to the dust cells and the epithelioid cells forming the tubercles in the lung is today abandoned.

COMMENT

From my observations it became apparent: (1) that the large mononuclear cells found in the pulmonary alveoli in inflammatory and congestive processes are macrophages; (2) that they originate from the cells found along the wall of the air vesicles known as respiratory epithelium. This observation is shared by the majority of modern pathologists (Aschoff, Bloom, Seemann, Marchand, Maximow, Carlton, Cappell, Lang, Pagel, Policard, Huguenin, Tchistovitch, Chiodi). The point of dispute is the nature of these cells, which are regarded by one group of observers (Aschoff and his school) as endodermal, and by others (Maximow, Lang, Bloom, Policard and myself) as mesodermal.

Seemann, as the result of experiments carried out in Aschoff's laboratory, related findings closely approaching my own, maintaining, none the less, that the cells lining the air sacs are of endodermal origin. He based his opinion on "classic" embryologic data and on the results obtained by staining tissues from lungs with silver nitrate. He did not discuss in detail the macrophagic traits of these cells, but dismissed the subject by saying that "the outstanding activity of the 'alveolar epithelium' should not surprise us if we remember that *occasionally* [italics mine] the liver, kidney, adrenal and testicular cells are strongly phagocytic." This statement cannot be relied on, since these cells are passive (facultative) phagocytes, whereas the alveolar "epithelium" is actively (obligatorily) phagocytic. This is of importance in connection with the statement made by Aschoff: "It is known that occasionally every possible cell will 'swallow' coarse foreign elements; i. e., it will react as a macrophage. . . . There is hardly a fixed cell in the

organism that is not in a position, under certain circumstances, to swallow and to digest other cells, foreign bodies and parasites. Are they thereby functionally identical? Not to the slightest degree. There is concerned here one of the functions proper to every cell, that is, of digestion. One must, therefore, also look for other characteristics. In the cell with which we are concerned (the reticulo-endothelial) phagocytosis is only one property which is particularly marked. The intensity and the amount of phagocytosis are here the deciding factors." Then, too, the "phagocytic" epithelial cells of the viscera mentioned by Seemann do not show proliferation as a result of "irritation" and do not transform themselves into epithelioid cells under the influence of the tubercle bacillus. They *occasionally* engulf foreign elements while still attached to their basal membranes, but are invariably found to be dead when detached from their customary seat, whereas the alveolar "epithelial" cells display ameboid phagocytic activities in space. Staining with silver nitrate, the method applied by Seemann to prove his thesis, is regarded by most observers to lead to ambiguous pictures. Finally, the nature of the structure of the fetal lung, which serves as an argument in support of the epithelial nature of the epithelial cells, is far from being definitely determined. Today, as in the time of Virchow, "the doctrine of embryologic leaflets is not yet clearly elucidated in all its details and . . . every new investigation along this line brings some modifications" (Virchow).

Embryology teaches that the air sacs of the embryo are lined with a single uninterrupted layer of cuboidal epithelial cells. It is assumed that as a result of the first respiratory effect some of these cells become abruptly flattened and some lose their nuclei altogether, leading to the formation of the anucleated platelike cells of the functioning alveolus. The abrupt flattening of the cuboidal cells following the first respiratory movement and the function of the anucleated cells have, however, never been plausibly explained. In fact, recent observers deny their existence. "Ce sont des 'êtres de raison' acceptés par habitude et par respect d'un schema traditionnel," according to Policard. ("These are 'creatures of reason' accepted by habit and out of respect for tradition.")

With regard to the abrupt flattening of the "respiratory epithelium," Fauré-Fremiet and Dragoiu found that in the sheep the transformation is not abrupt but occurs gradually, beginning in the second half of embryonic life, at which time free spaces appear between the alveolar cells. At this juncture the nucleated cells take on a new aspect and acquire new potentialities. These investigators observed, for instance, that the cells contain a chemical substance closely akin to glutathione described by Hopkins. "It is certain," stated Huguenin, Foulon and Delarue, as a result of experiments performed in Roussy's laboratory,

"that the cells which at one time were morphologically and physiologically epithelial are replaced by elements which are the future granular (foam) cells of the adult lung." Policard and Chiodi believed that the cuboidal cells lining the fetal alveoli completely disappear, thus "denuding" the respiratory sac, which is subsequently partly "relined" by immigrated mesenchymal cells. In this connection it may be of interest to note that during embryonic life the esophagus, too, changes its lining on five occasions. It also was observed that even in postembryonic life processes of involution of organs or structures occur. Recent studies by Smith and Bennet, made in Wolbach's laboratories, revealed that in postembryonic life the size and number of the alveolar cells in the rat vary with age.

Bloom reproduced in Maximow's textbook of histology two photographs of the lungs of an embryo guinea-pig near term to show the change which takes place in the embryonic lung when the air spaces become distended with fixing fluid. "It is clear," wrote Bloom, "that at this stage, even before injection of the fixative, there is no longer a cuboidal lining of the alveoli."

No systematic studies on the embryology of the lung were made by me. Casual observations with the use of the technic of insufflation of the fetal lung within physiologic limits (a method ordinarily used by me in experiments on postembryonic lungs) have revealed pictures hitherto unobserved. Thus, figure 5 *A* is a photomicrograph of a section of a lung removed from an embryo guinea-pig measuring 2.5 cm. in length. The picture illustrates the peculiar alveolar structure of the fetal lung in the early stages. In figure 5 *B* a section from the lung of an embryo guinea-pig 6.5 cm. long (near term) is shown. It may be seen that at this period the classic "adenomatous" structure no longer exists.

The observation on the imperfect lining of the air sacs was known to earlier observers, such as Zenker, Villemin, Todd and others. Claude Bernard, in studying the absorbing power of the mucous membrane of different organs, found that toxins and poisons are absorbed by way of the alveolar surface as rapidly as by way of the blood stream. He noticed that curare placed on the mucosa of the bronchi caused no harm to the animal, but that when this alkaloid was "pushed" into the alveoli it killed the animal as rapidly as if it had been injected into the circulation. He attributed this phenomenon to the lack of an "epithelium protecteur à la surface des vesicules pulmonaires." In a recent study Policard stated: "The respiratory surface of the lungs ought to be compared to an open wound."

In some pathologic conditions of the lungs structures resembling acini lined by cuboidal cells occur. These were interpreted as respiratory

alveoli lined by cuboidal cells that have reclaimed their ancestral embryonic form. I have observed that the glandular structures present in sclerosed lungs are often no other than newly formed alveolar structures

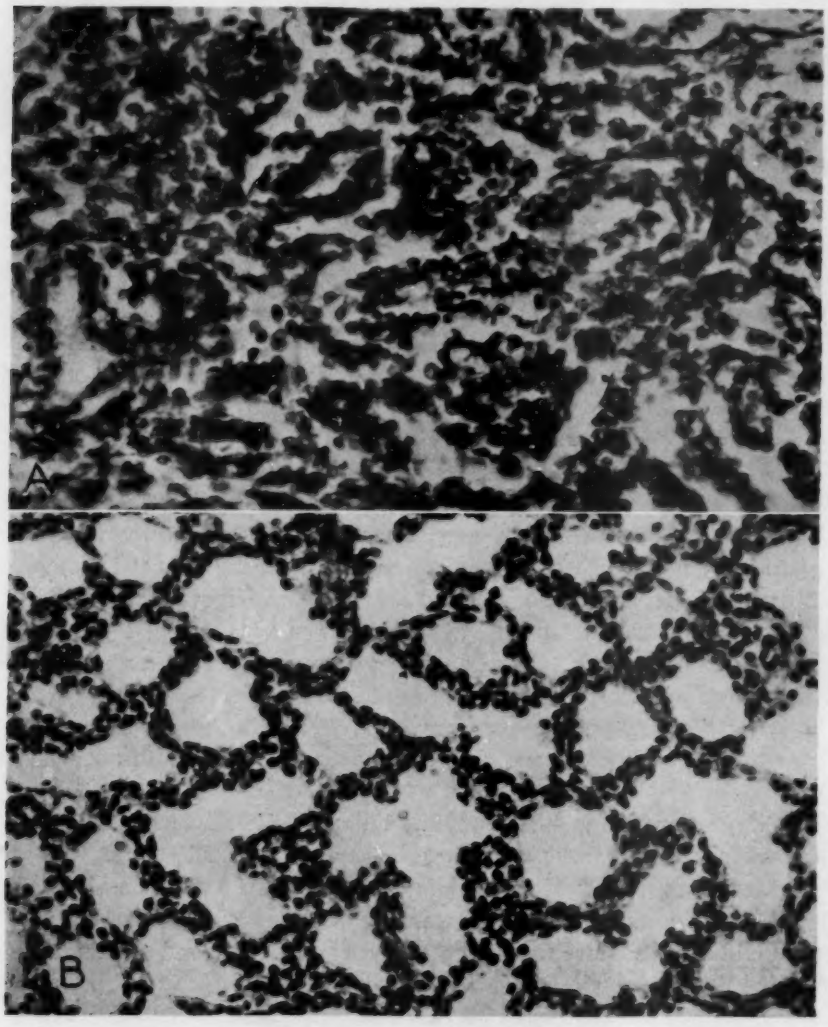

Fig. 5.—*A*, section of the lung of an embryo guinea-pig 2.5 cm. long. The glandlike structure should be noted. *B*, section of the lung of an embryo guinea-pig 6.5 cm. long, i. e., near term. The "glands" seen in the earlier stages of development no longer exist.

lined by cells of bronchiolar origin. Analogous pictures are observed in atrophic (Laennec's) cirrhosis of the liver, in which there also occurs a new formation of bile ducts within the rings of connective tissue

encircling the distorted hepatic lobules. In diffuse sclerogenic disorders of the lungs the respiratory alveoli, too, appear like glandular structures lined by an uninterrupted layer of cuboidal cells, an appearance which

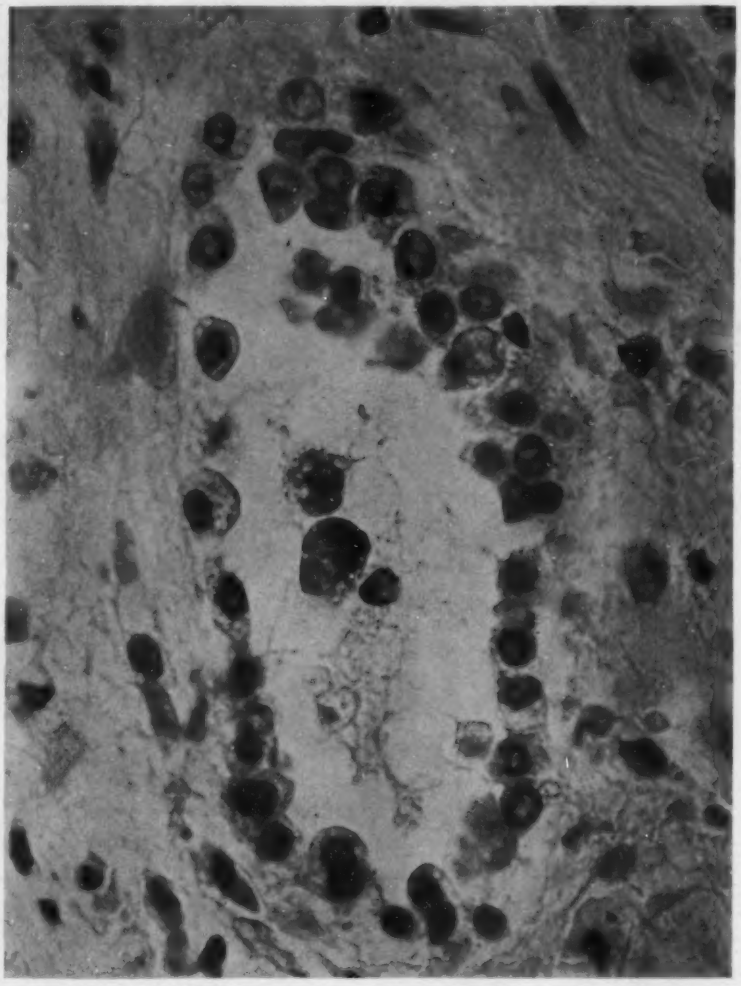

Fig. 6.—Lining of a pulmonary alveolus in a diffusely fibrosed human lung. The similarity of the sessile and the free cells, which have all the earmarks of macrophages, should be noted. Two free cells ("heart disease cells") contain blood pigment. Reduced from × 1,100.

has often been cited as evidence of the epithelial nature of the alveolar cells. However, a study of numerous lungs with similar pictures has convinced me that here, too, the cells of the alveolar wall behave in ways similar to that of macrophages and that their morphology is often

that of large mononuclear phagocytes, as is seen in figure 6. Similar pictures are observed in the sinuses of the spleen in diabetic lipemia, in Gaucher's disease, in Niemann-Pick's disease and also in cirrhotic conditions of the spleen. The splenic sinuses in these diseases have been often referred to as "cystic structures lined with cuboidal epithelium" (fig. 3).

Experimental and clinical observations point to the conclusion that the so-called epithelial cells which are found singly and in groups along

Fig. 7.—Cut surface of the lungs of a rabbit infected with bovine tubercle bacilli by way of the blood stream, showing diffuse miliary tuberculosis. The animal lived six weeks after the infection.

the septums of the pulmonary alveoli are probably of mesenchymal origin; that is to say, they are "resting" macrophages and accordingly produce cells of their kind, i. e., the "wandering" macrophages of the air sac. This hypothesis is also supported by the examination of pulmonary tissue inoculated with tubercle bacilli in vitro. Thus, Lang found that the alveolar phagocytes originate from the alveolar "epithelium," which he regarded as being polyblasts or macrophages. He emphasized that they are a counterpart of those present inside the sep-

tums. He likewise observed that the phagocytic cells which are found around the pulmonary vessels and bronchi are similar to the cells lying in and on the septums.

The behavior of the lungs in instances of infection by way of the trachea reveals that the respiratory portion of these organs possesses an exquisitely efficient defensive structure. It is remarkable that the

Fig. 8.—Cut surface of the lungs of a rabbit infected with bovine tubercle bacilli via the trachea. The lungs are markedly enlarged and show nearly complete caseation. The heart is pushed upward, and the upper lobe of the left lung is reduced in size as a result of compression. There is an adhesive fibrous pleuritis leading to obliteration of both pleural cavities. The animal lived four times longer than its fellow, the lungs of which are shown in figure 7. Both animals were of the same weight and age at the time when they were infected with the same amount of tubercle bicilli.

thin and "half naked" alveolar wall which has no more resistance than a "spider's web" in regard to fluids, is a virtual fortress when bacteria have penetrated into the respiratory portion of the lungs.

The difficulties encountered in inducing a pulmonary disease in animals infected via the trachea have been baffling observers since the advent of bacteriology. I have observed that a dose of pneumococci that kills a rabbit in about eighteen hours, when injected into the blood stream, causes the death of another rabbit infected by way of the trachea in about four days. On the other hand, a dose of this micro-organism sufficient to cause the death of a hematogenously infected animal within from three to four days is harmless to an animal infected via the respiratory tract. The micro-organism is promptly phagocytosed and destroyed by the alveolar "epithelial" cells. Likewise, in the resolution of lobar (fibrinous) pneumonia, the alveolar "epithelial" cells and not the granulocytes play the rôle of scavengers, performing this function by phagocytosis and also by secreting lytic substances. The granulocytes flood the lung in the early stages of the disease, being supplanted later by alveolar ("epithelial") phagocytes.

I have mentioned the differences existing between the hematogenous and the aerogenous infection of rabbits with the tubercle bacilli (figs. 6 and 7). Thus, the duration of the disease in animals infected with Koch's bacillus via the trachea is about four times longer than in those infected by way of the blood stream. The disease is disseminated throughout the body at a much later period in aerogenous infection, possibly in the terminal period, and in some instances it is confined for life to the lungs.

The efficacy of the pulmonary filter in regard to pathogenic bacteria was stressed by Besredka in the following terms: "Tant qu'il est intact, le filtre pulmonaire est imperméable à la façon d'une bougie à pores serrées; mais il suffit la lésion la plus minime pour que sa porosité augmente au point de rendre le filtre inopérant." ("So long as the pulmonary filter is intact it is as impermeable as a candle with tight pores. But the slightest lesion is sufficient to increase its porosity to such a degree as to render the filter ineffective.")

SUMMARY

Since the time of Magendie and Claude Bernard it has become known that of all mucous membranes that of the lungs possesses the best absorbing power. Thus, alkaloids or dyes injected into the lungs via the trachea are detected in the general circulation and in the urine within a few minutes after the injection.

On the other hand, since the advent of the bacteriologic era it became obvious that fine emulsions of pathogenic bacteria which are fatal to animals by intraperitoneal, subcutaneous or hematogenous infection are harmless when the respiratory portion of the lungs is chosen as an avenue for the infection.

The experiments reported in this review were undertaken in order to study the mechanism whereby the lungs get rid of the pathogenic invaders, thus maintaining their sterility and serving as a barrier against a systemic dissemination of the disease.

The studies have revealed that in the lungs, as elsewhere in the organism, the protective function lies essentially in the macrophages, which dispose of the invader by means of phagocytosis.

It was further demonstrated that in the early phases of pulmonary infection these cells are not imported from outside sources but are abundantly supplied by the lungs themselves.

It was shown, moreover, that most of the wandering macrophages of the pulmonary alveoli originate from the cells lining the air sacs. The nature of these cells was analyzed in some detail, and evidence was produced to show that very likely they are not epithelial but mesenchymal in origin. Experimental evidence was cited to the effect that these cells, and not others, are concerned in the defensive and metabolic processes in which the lungs are involved, and that they act in ways similar to the Kupffer cells, the splenocytes and the reticular cells of the bone marrow and the lymph nodes.

It is believed that they represent an essential part of the macrophage system.

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News and Notes

Grants-in-Aid.—Jean R. Oliver, professor of pathology at the Long Island College of Medicine, has been assigned an additional grant of $1,000 by the Josiah Macy, Jr., Foundation for continued studies on the pathology of Bright's disease.

The committee on grants-in-aid of the National Research Council at its December meeting, in response to 119 requests, made 36 grants for the support of research projects, of which the following 6 come under the heading of medical sciences: G. Howard Bailey, Johns Hopkins University, "Heterophile Antigens of Bacteria and Plant and Animal Tissues"; Raymond L. Garner, Johns Hopkins University, "Enzymatic Liquefaction of Clotted Human Blood"; R. W. Gerard, University of Chicago, "The Activity of Nerve Tissue and the Central Nervous System"; Balduin Lucké, University of Pennsylvania, "A Neoplastic Disease of the Common Leopard Frog, *Rana Pipiens*"; John R. Paul and James D. Trask, Yale University, "Comparison of Different Strains of Poliomyelitis Virus"; Arthur H. Smith, Yale University, "The Influence of Various 'Inorganic' Ions upon the Body Weight and Blood Changes of Experimental Animals."

A New Tumor Registry.—A tumor registry has been established by the medical society in Peoria, Ill. Any physician who registers a case may become a member. For the present all records will be kept in the laboratory of the St. Francis Hospital.

Prize Paper.—The eleventh annual prize of $1,000, given by the American Association for the Advancement of Science to the author of a noteworthy paper presented at the winter meeting, was awarded at Boston to Dr. Reuben L. Kahn, professor of bacteriology at the University of Michigan Medical School, for his paper, "Tissue Reactions in Immunity."

Society News.—The American Public Health Association will hold its sixty-third annual meeting in Pasadena, Calif., from September 3 to 6.

The forty-sixth annual meeting of the American Physiological Society, under the presidency of Arno B. Luckhardt, will be held under the auspices of the College of Physicians and Surgeons, Columbia University, from March 28 to 31. The scientific meetings will be held in the Hotel Pennsylvania, which will also serve as headquarters. The demonstrations will be made at the College of Physicians and Surgeons.

Officers of the American Society of Parasitologists have been elected as follows: president, E. E. Tyzzer, Harvard Medical School; vice-president, J. E. Ackert, Kansas State College; secretary, H. W. Stunkard, New York University; treasurer, J. Andrews, Johns Hopkins University School of Hygiene and Public Health.

The Pathological Society of Philadelphia has elected officers for 1934: Morton McCutcheon, president; Esmond R. Long, vice-president; Herbert L. Radcliffe, secretary-treasurer.

The annual meeting of the American Association of Pathologists and Bacteriologists will be held in Toronto, Thursday and Friday, March 29 and 30, 1934. One-half day will be given to a joint meeting with the American Association of Immunologists.

CLEVELAND SESSION—AMERICAN MEDICAL ASSOCIATION

Scientific Exhibit

The Section on Pathology and Physiology of the American Medical Association will sponsor a section exhibit in the Scientific Exhibit at the Cleveland Session, June 11-15, 1934. The section exhibit committee is composed of: W. C. MacCarty, chairman, Rochester, Minn.; Frank W. Hartman, Detroit; A. B. Luckhardt, Chicago, and J. P. Simonds, Chicago.

Applications for space will close on Feb. 26, 1934. Application blanks may be obtained from the members of the committee, or from the Director, Scientific Exhibit, American Medical Association, 535 North Dearborn Street, Chicago.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

SYNOVIAL CELLS IN TISSUE CULTURES. E. VAUBEL, J. Exper. Med. **58**:63 and 85, 1933.

Synovial cultures are differentiated from cultures of other tissues of mesenchymal origin by the type of growth and the function of the cell. In these respects, synovial cultures are more closely allied to chondroblasts and osteoblasts than to fibroblasts. Synovial cells in tissue cultures acquire marked globular cytoplasmic granulations that stain easily with neutral red and sometimes with toluidine blue; they show marked polymorphism with all transitions from round to spindle, polygonal and star shapes, and eventually form an epithelium-like membrane, composed of cells with numerous syncytial bridges. In cultures of typically growing synovial cells a mucin-like substance is elaborated. Typical growth and maximum production of mucin are best maintained in mediums containing a minimum of growth-stimulating substances. Transformation of synovial cell growth into fibroblastic growth is accompanied by a loss of production of mucin. Dying cells apparently do not produce mucin. Amitotic cell division and the formation of macrophage-like cells were observed. A marked tendency to liquefaction of the plasma about the growths was observed and attributed to the elaboration of a proteolytic ferment. The specific designation "synovioblasts" is proposed for these cells.

AUTHOR'S SUMMARY.

TISSUE CULTURES OF FIBROBLASTS. R. C. PARKER, J. Exper. Med. **58**:97, 1933.

Under appropriate conditions, fibroblasts are able to multiply in serum, at a slow rate, for long periods. The rate of multiplication of fibroblasts in a given sample of serum depends entirely on the nature of the strain. Cell races endowed with a high residual growth energy multiply more rapidly in serum than those whose growth potencies are of a lower order. Fibroblasts in the beginning multiply more abundantly in plain serum than in heparinized serum and more abundantly in heparinized serum than in heparinized plasma. Later, these differences become less pronounced. The first effect of serum on fibroblasts is invariably injurious, the degree of injury differing according to the nature of the cell strain and the age of the animal from which the serum is derived. With the passage of time, however, the colonies undergo gradual improvement both in the appearance of the component cells and in their rate of proliferation. In mediums containing the embryonic tissue juice or other growth-activating substances, fibroblasts form colonies that are isomorphic and composed of isomorphic cells. In serum, fibroblasts form colonies of heteromorphic appearance. Each colony becomes composed of cells that differ from one another to a more or less marked degree.

AUTHOR'S SUMMARY.

PREVENTION OF CHOLESTEROL ATHEROSCLEROSIS IN RABBITS. K. B. TURNER and G. B. KHAYAT, J. Exper. Med. **58**:115 and 127, 1933.

In seventeen of nineteen animals, whole thyroid gland, when administered simultaneously with cholesterol, prevented the atheromatous changes produced by the latter in the aorta of rabbits. In this series, thyroxine was less effective, as atherosclerosis occurred in eight of eleven rabbits. Potassium iodide also exerted a strong protective action, as aortic lesions were present in only one of a series of twelve rabbits fed cholesterol and potassium iodide concurrently. The effectiveness of potassium iodide was not shared by potassium bromide or potassium chloride.

A relationship was noted between the level of the cholesterol in the blood and the development of atherosclerosis. In general, the aortic lesions accompanied hypercholesteremia.

Thyroidectomy in itself does not cause a rise in blood cholesterol or the development of atherosclerosis in young rabbits. The feeding of cholesterol produces hypercholesteremia and atherosclerotic lesions in rabbits regardless of the presence or absence of the thyroid glands. Potassium iodide prevents the usual hypercholesteremia and atherosclerosis of the aorta in normal rabbits fed cholesterol, but when the thyroid glands are removed this protective action disappears.

AUTHORS' SUMMARIES.

ARTERIAL DISEASE PRODUCED BY CHOLESTEROL AND VITAMIN D. C. V. HARRISON, J. Path. & Bact. **36**:447, 1933.

In experiment 1, cholesterol sclerosis was induced in the rabbit's aorta as a preliminary lesion, and vitamin D sclerosis was later superimposed. In experiment 2, vitamin D sclerosis constituted the preliminary lesion, and cholesterol sclerosis was added. In both cases, the second lesion was confined to the parts of the vessel left unaffected by the first lesion. Both cholesterol sclerosis and vitamin D sclerosis render the affected parts of the vessel wall relatively immobile, and this immobility appears to render the walls less susceptible to the subsequent lesion. It is concluded that the movements of the vessels determine the localization of the lesions due to lack of vitamin D as well as those due to cholesterol, and probably those due to human arterial disease.

AUTHOR'S SUMMARY.

ATHEROSCLEROSIS IN DOGS. W. D. ZINSERLING, Beitr. z. path. Anat. u. z. allg. Path. **88**:241, 1932.

The aorta and other organs of twenty-eight dogs, from 8 to 28 years old, were studied. In old dogs, spontaneous lipoidosis of the aorta, the interstitial tissue in the sclera, cartilage, tendons and the fibrous capsule of the internal organs was observed. The lipoid was deposited in the interstitial substance, while the fatty degeneration of the tissue cells played a minor rôle. These changes were identical with those observed in man and the rabbit. Characteristic for the dog is the secondary lipoidosis in fibrotic areas of the intima, in contradistinction to the primary lipoidosis of the human aorta. In man and in the rabbit, fibrosis of the aortic wall follows atheromatous degeneration. In the dog, cholesteremia does not play the same important rôle of a causative factor of lipoidosis of the aorta as it does in the rabbit. It was impossible to produce lipemia and atherosclerosis in dogs by feeding an excess of cholesterol. In old dogs, endogenous factors seem to produce a disturbance of the cholesterol metabolism which, together with the fibrosis of the aortic wall, must be regarded as the essential cause of atherosclerosis.

C. ALEXANDER HELLWIG.

HEMATOPOIESIS IN THE AXOLOTL AFTER REMOVAL OF THE EXTERNAL ZONE OF THE LIVER. B. T. MALYSCHEW, Beitr. z. path. Anat. u. z. allg. Path. **88**: 315, 1932.

In the axolotl, formation of the granulocytes occurs normally in the external zone of the liver, while the spleen produces the red blood cells. After removal of the external zone of the liver, the reticulo-endothelial cells lining the muscle trabeculae of the heart and the Kupffer cells of the liver produce hemocytoblasts. The latter develop into granulocytes and erythroblasts. Some of the newly formed hemocytoblasts are carried to the epicardium and form there a marginal zone of hematopoiesis, with neutrophil and eosinophil granulocytes predominating. The external zone of the liver regenerates from Kupffer's cells and their descendants, the hemocytoblasts, whereas the parenchyma proper of the liver is regenerated by mitotic division of liver cells.

C. ALEXANDER HELLWIG.

THE INFLUENCE OF FOOD ON THE HEALING PROCESS OF WOUNDS IN THE STOMACH. E. L. HOWES, Beitr. z. path. Anat. u. z. allg. Path. **88**:435, 1932.

The healing of surgical wounds in the stomach was studied in three groups of white rats. After operation the first series received a well balanced diet; the second series, one half of the volume and of the caloric value given the first, and the third series, three fourths of the caloric value of the stock diet, the volume being brought up to normal by the addition of sawdust. A diet containing only half of the regular volume and half of the caloric value did not interfere with the process of healing. In the third series, however, the process of healing was definitely retarded. Since this group of white rats received a diet which was of higher caloric value and more voluminous than that of the second group, the irritating sawdust in the diet must have been the responsible factor. Postoperative diet, therefore, should be completely free from irritating substances.

C. ALEXANDER HELLWIG.

CONSTITUTIONAL THROMBOPATHY (A NEW DISEASE OF THE BLOOD). E. A. WILLEBRAND and R. JÜRGENS, Klin. Wchnschr. **12**:414, 1933.

The authors studied thirty-five "bleeders" from a group of three families with bleeders for four generations. The results are believed to justify the establishment of a new group in the classification of diseases of the blood. Constitutional thrombopathy is a dominant, sex-linked, hereditary disease, occurring more frequently in females than in males and characterized by spontaneous bleeding from the skin, mucous membranes and wounds. The blood platelet count is normal. However, the bleeding time is markedly prolonged. The plasma proteins are normal. The authors ascribe the disease to hypofunction of the blood platelets, and particularly to a diminution in the agglutination of thrombocytes and deficiency in thrombus formation. The condition is distinct from hemophilia and thrombopenia.

J. KLEIN.

DIAPHRAGMATIC ANGINA PECTORIS. L. HOFBAUER, München. med. Wchnschr. **80**:411, 1933.

The author is of the opinion that attacks of angina pectoris may be due to diseases of the diaphragm, such as hernia and empyema, and cites clinical records to support his contention. Hyperesthesia of the diaphragm may cause pain, dyspnea and a feeling of constriction in the chest. When there is no involvement of the diaphragm, the diaphragm may nevertheless become hyperesthetic as the result of changes in the circulatory organs (the coronary arteries, aorta and myocardium) caused by a visceromotor reflex.

J. KLEIN.

ORIGIN OF BONE MARROW GIANT CELLS IN EXTRAMEDULLARY MYELOPOIESIS. R. P. CUSTER, Virchows Arch. f. path. Anat. **288**:213, 1933.

In a series of experiments undertaken to study the effect of reticulo-endothelial blockade on extramedullary myelopoiesis, marked formation of bone marrow giant cells in the spleen was noted in one animal. In lesser degree the phenomenon was also evident in the liver and the lymph nodes. In this animal, india ink had been used to form the blockade and sapotoxin to cause anemia. The bone marrow appeared inactive and degenerated. In another rabbit that had also received sapotoxin, but in which carmine had been used for blockade, the bone marrow was very active and contained many hyperplastic giant cells. The spleen, liver and lymph nodes contained few giant cells. The author interprets his findings as indicative of the local extramedullary formation of the giant cells and postulates the action of a necrohormone as a factor in the process.

O. T. SCHULTZ.

LOCAL CIRCULATORY REACTIONS OF THE SKIN. E. REINHARDT and G. RICKER, Virchows Arch. f. path. Anat. **288**:393, 1933.

The effects of local mechanical and chemical stimuli on the vessels of the skin, as determined by capillary microscopy, form the basis of an extended theoretical discussion of the resulting changes and their interpretation and of a critical survey of the existing theories relied on to explain the phenomena noted. The various stimuli, regardless of their nature, evoke a diphasic reaction, which consists in ischemic anemia followed by peristaltic hyperemia of the papillary vessels. The hyperemia of stronger stimulation may lead to exudation and the formation of wheals. Maximal stimulation leads to stasis without exudation. The various reactions are the expression of the degree of action and not of the quality of the stimulus. They result from the action of the stimulus on the constricting and dilating nerves of the vessel. The action may be either direct or indirect, i. e., reflex. The place of action may be any part of the nervous system from the local vessel to the cerebral cortex, and the direction of the stimulus may be either centripetal or centrifugal. The stimulus which manifests itself in a local vascular reaction of the skin may therefore be psychic, mechanical, physical or chemical. Whatever the character of the stimulus, the vascular response is of the same character, varying only with the degree of action of the stimulus. The older cellular theory of vascular physiology, which postulates an action directly on the cells of the vessels, is held to be untenable. The more modern doctrine of humoral vascular physiology according to which soluble substances in the blood or tissue fluids mediate the vascular reactions is also held to be untenable. O. T. SCHULTZ.

FUNCTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL STREPTOCOCCIC SEPSIS. J. VOICU, A. VITÁLYOS and L. BOER, Virchows Arch. f. path. Anat. **288**:455, 1933.

Rabbits were infected with streptococci isolated from puerperal fever. In overwhelming, rapidly fatal experimental infections, there was no formation of opsonins, agglutinins or bacteriotropins. The reticulo-endothelial system of the animals revealed no reactive changes, and its cells had only weak properties of vital storage. In less severe infections, opsonins, agglutinins and bacteriotropins appeared in the blood. In animals killed during this state, the reticulo-endothelial system revealed hypertrophy and hyperplasia of its cells, which exhibited greater activity for vital storage than is normal. The formation of immune bodies and the hyperplasia and hyperactivity of the reticulo-endothelial system cease when the resistance of the animal is overcome by the infecting organism. The authors conclude that the study of the reactions of the reticulo-endothelial system is an excellent method for evaluating the therapeutic efficiency of remedial agents.

O. T. SCHULTZ.

TOXIC EFFECTS OF IRRADIATED STEROLS. B. KELLNER, Virchows Arch. f. path. Anat. **288**:491, 1933.

The histologic effects of irradiated sterols were studied in two series of fully grown young rats comprising, respectively, 85 and 130 animals. It is of interest that crystalline viosterol was found to be nontoxic in the first series of animals, although the substance is therapeutically active in rickets in man. This led to a study of the daily dosage of several irradiated sterols of ergot origin necessary to cause toxic effects. Subtoxic doses caused no histologic changes, even when continued for a long time, to half a year. The toxic effects noted were those previously described, namely, degeneration and necrosis followed by calcification. The kidney and aorta were most frequently affected. The peripheral arteries, heart muscle and voluntary muscle came next. In the bones toxic doses led to the formation of osteoid tissue, followed in some animals by hypertrophic changes in the bones and in others by atrophic changes, the latter being noted especially with the larger doses or a more prolonged administration. O. T. SCHULTZ.

RELATION BETWEEN THE ENDOCRINE GLANDS AND THE ESTRUS CYCLE. S. TSUCHIMOTO, Jap. J. Exper. Med. **11**:129, 1933.

The subcutaneous transplantation of the anterior lobe of the hypophysis of the guinea-pig or the rabbit caused ovulation in immature white rats. The genital organs showed early maturity; ovarian follicles developed and matured, changed into corpora lutea or formed cysts. The anterior lobe had no influence on ovulation in maturity. The central and posterior lobes caused no change in the estrus cycle, and the anterior lobe failed to prevent shrinkage of the genital organs after ovariectomy.

J. KLEIN.

Pathologic Anatomy

DEGENERATION OF THE CORPUS CALLOSUM IN HYDROCEPHALUS. N. A. ZOLOTOWA, Virchows Arch. f. path. Anat. **280**:343, 1931.

In two cases of internal hydrocephalus (one syphilitic, the other congenital) in infants the corpus callosum was found to be markedly atrophied. Primary failure of development and atrophy secondary to the hydrocephalus are possible causes. However, the anlage of this structure develops after the fourth month of pregnancy. The occurrence of internal hydrocephalus before this time interferes with its development, resulting in a rudimentary type.

PERRY J. MELNICK.

ACROCEPHALOSYNDACTYLISM (APERT). ARNOLD FLINKER, Virchows Arch. f. path. Anat. **280**:546, 1931.

The author reviews the nine cases of acrocephalosyndactylism gathered from the literature by Apert, and adds a tenth. The condition is characterized anatomically by syndactylism of all four extremities and a characteristic deformity of the head consisting of acrocephaly, a peculiar outline of the forehead and flattening of the occipital region. There are other variable congenital anomalies. The condition is not hereditary or familial. The author's patient came from a cretin population and had a large goiter and myxedema.

PERRY J. MELNICK.

LIPOID NEPHROSIS. A. R. KANTROWITZ and PAUL KLEMPERER, Virchows Arch. f. path. Anat. **280**:554, 1931.

Using histologic methods recommended by McGregor which bring out finer morphologic changes than does the hematoxylin-eosin stain, Bell came to the conclusion that lipoid nephrosis is primarily glomerulitis. The authors studied two cases of undoubted clinical and pathologic lipoid nephrosis by these methods. Proliferation of glomerular endothelium or of capsular epithelium, the criterion of glomerular inflammation, could not be found. The cells of these structures were swollen and contained lipoid droplets, changes which were not so definite with the hematoxylin and eosin stain. However, these changes are considered not to be inflammatory but to be phenomena of degeneration and lipoid storage, part of the picture of lipoid nephrosis.

PERRY J. MELNICK.

CHANGES IN THE ELASTIC TISSUE OF THE LUNG IN A CASE OF BRONCHIAL ASTHMA. W. WAWERLA, Virchows Arch. f. path. Anat. **285**:12, 1932.

In a man, aged 36, who had had bronchial asthma for twenty-four years, histologic examination of the lungs revealed some unusual changes in the elastic tissue. The elastic fibers of the terminal arterioles and of the smaller bronchi were ruptured, swollen and encrusted with iron and calcium. About many of the arterioles there had been formed foreign body granulomas that compressed and occluded the vessels. In the pulmonary parenchyma the elastic tissue framework was in places hypertrophied and in other places broken and torn, and in still other areas elastic tissue had disappeared. Occlusion of the small arteries had led to

hypertrophy of the right side of the heart. Engorgement of the vessels and pathologic functioning of the lung are held responsible for the changes in the elastic tissue.

O. T. SCHULTZ.

UNUSUAL FATAL INTESTINAL HEMORRHAGE. H. WEBER, Virchows Arch. f. path. Anat. **285**:46, 1932.

A woman, aged 50, was found, a year before her death, to have tertiary syphilids of the skin and cerebrospinal syphilis that yielded to antisyphilitic treatment. Syphilitic cirrhosis of the liver developed, and she died from a sudden massive intestinal hemorrhage. At necropsy there was found a ruptured submucous varix of the lower portion of the ileum. This portion of the intestine was adherent in a hernia of the scar of a previous laparotomy. The unusual situation of the ruptured varix was due to the interference of the adhesion with the collateral circulation, which everywhere else was well developed.

O. T. SCHULTZ.

QUANTITATIVE STUDY OF OVARIAN FOLLICULAR ATRESIA. W. BLOTEVOGEL, Virchows Arch. f. path. Anat. **285**:53, 1932.

By means of serial sections of both ovaries of sixteen white mice and five primates (Cynocephalus hamadryas), the total number of primary, secondary and atretic follicles was determined and the volume of follicular tissue in relation to the volume of the entire ovary was estimated. In both the species studied there was great variation in the number of follicles in animals of the same species, in the number of follicles in the two ovaries of the same animal and in the size of the ovaries. In each species about half of the follicles were undergoing atresia. In the primate there was a correlation between the number of follicles and the volume of the ovary; in the mouse no such correlation could be detected. In neither species was there a correlation between the number or the volume of atretic follicles and the volume of the ovary, indicating that follicular atresia is not the result of overcrowding and lack of space within the ovary. This conclusion forms the basis of a brief discussion of inherent lethal factors within the germ cell, as opposed to environmental factors. The inherent lethal factors manifest their effects even before fertilization. In the species studied, approximately half of the germ cells are destined to destruction within the ovary.

O. T. SCHULTZ.

CONGENITAL MALDEVELOPMENTS OF THE NOSE. B. LANG, Virchows Arch. f. path. Anat. **285**:93, 1932.

Two unusual examples of nasal maldevelopment are described, one of proboscis lateralis and one of Kundrat's cebocephalic form of arhinencephaly. In each there were multiple malformations in addition to the nasal one, to which special attention is given. In the case of proboscis lateralis, the left half of the nose was normally formed. The right half was represented by a short, sessile structure attached at the inner canthus of the right eye. The right nasal bone and the right nasal cavity were absent. There was a coloboma of the right lower eyelid. The intermaxillary bone was absent. In the case of cebocephaly a rudimentary nose with a single nostril had been formed. The following structures had not been formed: the horizontal and vertical plates of the ethmoid, the small wings of the sphenoid, the optic foramina, the vomer, the lacrimal bones, the intermaxillary bone and the nasal cavities. The frontal lobes of the brain were fused in the midline, with maldevelopment of the olfactory lobes and other parts of the brain. The endogenous and exogenous factors that may have a part in the genesis of such maldevelopments, and the period of embryologic development at which the latter may arise, are discussed.

O. T. SCHULTZ.

CONGENITAL VERTEBRAL DEFORMITY WITH ABSENCE OF THE NECK. A. FELLER and H. STERNBERG, Virchows Arch. f. path. Anat. **285**:112, 1932.

In the fourth of a series of studies of congenital malformations of the vertebral column, the authors describe six cases of absence or shortness of the neck. Five of these occurred in fetuses and one in a woman who died at the age of 51 years. The anomaly was described by Kippel and Weil in 1912 and is referred to in the German literature as the Kippel-Weil syndrome. In the authors' cases there were various malformations of the cervical and upper thoracic vertebrae, including failure of development or rudimentary development with fusion of vertebrae. Common to all was a defect of the dorsal arches of the vertebrae, which continued upward into the occipital bone. The anomaly is ascribed to interference with the normal development of the primitive segments of the body; it manifests itself before cartilage has been formed in the anlagen of the vertebrae.

O. T. SCHULTZ.

HISTOLOGY OF UNDULANT FEVER. F. WOHLWILL, Virchows Arch. f. path. Anat. **286**:141, 1932.

A woman, aged 67, died suddenly at the end of the fourth week of an attack of undulant fever. Death was due to pulmonary emoblism; the embolus came from a thrombus of the femoral vein. The spleen was enlarged. Microscopic examination revealed numerous minute cellular nodules composed of epithelioid reticulo-endothelial cells. Intermingled with these were a few giant cells and a few plasma cells. The small granulomas were situated in the malpighian bodies, only a few being found in the pulp. The para-aortic and bronchial lymph nodes and the bone marrow contained similar minute granulomas. In the liver were numerous focal necroses, about some of which there was a granulomatous reaction similar to that of the spleen and lymph nodes. In the small number of cases of undulant fever in which necropsy has been performed, death has been due to some other condition and no changes characteristic of infection with Bacillus abortus have been noted. In Wohlwill's case also death was due to a condition other than the infection, but it occurred at a time when lesions that the author considers characteristic of the disease were still present.

O. T. SCHULTZ.

INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM IN A CASE OF GENERALIZED XANTHOMATOSIS (SCHÜLLER-CHRISTIAN'S DISEASE). H. CHIARI, Virchows Arch. f. path. Anat. **288**:327, 1933.

Previous reports of Schüller-Christian's disease have presented clinical or anatomic evidence of only slight involvement of the central nervous system. Chiari reports a careful histologic study of the brain in a case of the disease with marked involvement of the central nervous system. A man who died at the age of 27 had had osteomyelitis of the right tibia at the age of 20. Operation was followed by recovery. Five years before death the symptoms of diabetes insipidus appeared and persisted until death. Four years before death areas of softening were noted in the occipital bone; roentgenologic examination of these areas a year before death led to a clinical diagnosis of Schüller-Christian's disease, which was confirmed by biopsy. Headache, weakness of the extremities, difficulty in standing and walking, abnormalities of sight and hearing and projectile vomiting indicated severe and progressive involvement of the central nervous system. Death was due to cavernous pulmonary tuberculosis. Necropsy and the subsequent microscopic examination brought to light no areas of the characteristic cholesterol-infiltrated tissue in the thoracic or abdominal organs. Such tissue was, however, present in the ilium, femur, cranium, dura, brain and cervical cord. In the brain the xanthomatous tissue formed macroscopically visible yellowish areas in the cerebrum and cerebellum. These areas were situated in the white matter, chiefly about the ventricular system, and spared the gray matter of the cerebrum. The cortex of the cerebellum was involved. The yellow tissue was composed of vacuolated

foamy cells the cytoplasm of which contained doubly refractile droplets that stained with sudan III. The cells were of two types. Larger cells were of mesenchymal origin and were derived from the tissue about the blood vessels. Smaller lipoid-containing cells were derived from the microglia and oligodendroglia. The macroglia did not participate in the process. In the areas of xanthomatous tissue the myelin sheaths and axis cylinders had disappeared. Except in the cortex of the cerebellum and the anterior horns of the cervical cord, the ganglion cells contained no lipoid.

O. T. SCHULTZ.

HISTOPATHOLOGY OF THE MARGINAL NODULES CAUSED BY PROSTHESIS OF THE LOWER LIMB. F. WOHLWILL, Virchows Arch. f. path. Anat. **288**:576, 1933.

Zur Verth first described nodules that develop in the skin of the inner surface of the thigh where the margin of an artificial lower extremity makes pressure. Arising subepidermally, they lead to ulceration of the covering epidermis and to the formation of small abscesses, which may persist as chronic fistulas. According to the histologic studies of zur Verth and others, the lesions are foreign body granulomas that develop about fibers of leather that are forced into the skin by pressure. Wohlwill examined histologically twenty lesions of the kind under consideration. In three the granulomas contained bodies the foreign character of which could not be definitely determined; origin from the stroma of clumped red corpuscles is considered possible. In the remaining cases foreign bodies surrounded by giant cells were present, but in only one of these was the foreign body held to be actually foreign to the tissue. In the other instances the genesis of the foreign bodies could be traced to the hornified epithelium of hair follicles, which had undergone hypertrophy and hyperkeratosis as the result of pressure and moisture.

O. T. SCHULTZ.

CHRONIC ABSCESS OF THE THYROID. K. STOJALOWSKI, Virchows Arch. f. path. Anat. **288**:660, 1933.

The avascular character of the capsule protects the thyroid against infection by direct continuity from adjacent tissues. Infection of the gland usually occurs by way of the blood or lymph stream. In one of the two cases here reported the abscess was single and was surrounded by a thick fibrous wall. The infection originated in the tonsil. In the second case the abscesses were multiple, were surrounded by fibrous tissue and were secondary to an ulcerated epithelioma of the larynx.

O. T. SCHULTZ.

RELATION OF MICROLITHS IN THE BILE TO CHANGES IN THE LIVER AND GALLBLADDER. W. BÜTTNER and G. LEMMEL, Virchows Arch. f. path. Anat. **288**: 682, 1933.

Bile removed from the gallbladder at necropsy was examined microscopically for microliths. The attempt was made to correlate the occurrence of the latter with histologic changes in the liver and gallbladder. In 800 consecutive necropsies microliths were detected 75 times (9.4 per cent). The incidence of their occurrence increases with age and stasis or stagnation of bile, but simple mechanical stagnation is not a factor in their formation. Inflammation of the gallbladder with resulting stagnation of bile may lead to the formation of microliths. There was no parallelism between cholesterol infiltration of the mucosa of the gallbladder and the formation of microliths. The formation of microliths was associated most often with pathologic alterations of the liver cells of variable degree, but there is no uniform pathologic background for this formation. Microliths were seen more often in light-colored bile than in deeply colored specimens, and more often when the quantity of bile in the gallbladder was small than when it was large, indicating that disturbance in biliary secretion is a factor in the formation of microliths.

O. T. SCHULTZ.

RELATION OF RHEUMATIC INFECTION TO ARTERIOSCLEROSIS OF THE AORTA. M. SCHULZ and F. KLINGE, Virchows Arch. f. path. Anat. **288**:717, 1933.

The thirteenth contribution of Klinge and his co-workers to the pathologic changes in rheumatic infection is a profusely illustrated article of sixty-three pages that attempts to evaluate the rôle of rheumatic infection in arteriosclerosis of the aorta. The vascular pathologic process of the active stage of the disease was described in the fourth communication of the series. The present article begins with a brief description of these early changes in five additional cases in which the patients died while active rheumatic lesions were still present. It then proceeds with its main thesis, a study of the relation of aortic arteriosclerosis to rheumatism and a variety of other diseases. For this study 188 aortas were selected that revealed macroscopic evidence of arteriosclerosis. Each aorta was examined microscopically throughout its length by being rolled into a spiral and embedded in a single pyroxylin block. For special histologic studies smaller blocks were excised and examined in frozen and paraffin sections. According to the gross pathologic and clinical manifestations of the disease process, the material is divided into the following ten groups: (1) 12 cases of endocarditis or lesions of the joints of certain rheumatic origin; (2) 15 cases of endocarditis of probable rheumatic origin; (3) 24 cases of nonrheumatic endocarditis; (4) 20 cases of syphilis; (5) 10 cases of chronic infection of various kinds; (6) 26 cases of clinical hyperpiesis; (7) 19 cases of acute infection; (8) 7 cases of septicopyemia; (9) 13 cases of tuberculosis; (10) 43 cases of diffuse atheromatosis and arteriosclerosis of the aorta. For each group a tabulation gives briefly the gross characteristics of the aorta, the localization of the process and the changes in the media and adventitia for each case. Then follows a more detailed description of selected cases, which is succeeded by a succinct summary of the findings for the group. At the end of the article is a brief discussion of the relation of the findings to some moot problems in the genesis of arteriosclerosis. The focal character of the lesions of the media in many of the groups is accepted as evidence of primary damage to the media, with secondary hyperplasia and later degeneration of the intima. But the occurrence of swelling and fibrinoid degeneration of the ground substance of the intima, with no underlying medial alteration, is sufficient proof that the arteriosclerotic process may be initiated also by primary disease of the intima. Severe damage to the media occurs not only in syphilis, but in rheumatism and in a variety of other chronic diseases. Aortic disease of rheumatic origin may involve any part of the aorta, but was noted most frequently in the abdominal aorta. In rheumatism and in nonrheumatic chronic endocarditis, foci of lymphocytic infiltration and vascularized scars were frequently seen in the media. Acute infection of any kind may activate such older lesions of the aorta. Mucoid degeneration of the media was noted in a wide variety of chronic diseases, but was most marked in rheumatism. Deposition of calcium occurred in a wide variety of diseases, but was more marked in tuberculosis than in any other group. Streaks of avascular fibrosis in the media without any inflammatory reaction were seen most often in the aortas of the patients who had had high blood pressure. In tuberculosis there was not seen the inflammatory involvement of the media that was so frequently observed in rheumatism and other chronic diseases. The diagnosis of rheumatic aortic sclerosis may be made at the postmortem table from the gross appearance of the aorta, the localization of the process and a history of rheumatic infection. Even if one is unwilling to accept all the deductions and conclusions that Klinge has drawn from the work presented in this series of contributions, one must admire the volume of work on which the conclusions are based, the thoroughness with which the work has been done, the planned orderliness of the work and the concise, organized manner in which the work is presented in printed form.

O. T. SCHULTZ.

UNUSUAL HISTOLOGIC FORMS OF RHEUMATIC INFECTION. R. RÖSSLE, Virchows Arch. f. path. Anat. **288**:780, 1933.

Rössle begins with a discussion of the question whether rheumatic infection is a disease of one or more organs, which is the older view, or a systemic disease of the mesenchyme involving widely the connective tissue and blood vessels, which is the concept of Klinge and other contemporary observers. The widespread involvement of the mesenchymal system of tissues can be explained only on the basis of an allergic state of these tissues. That febrile rheumatism is a disease characterized by hyperergic inflammation of the mesenchyme Rössle considers established. Variations in the degree and localization of the hyperergic reaction depend, in part, on differences in the degree of sensitization of the mesenchyme, on differences in the physiologic function of the connective tissue and blood vessels of the organs and tissues and on differences in the mechanical activity of the tissues. As a contribution to the conception that rheumatic infection is a mesenchymal systemic disease, he describes and discusses in detail five cases that differed widely from each other in their clinical and pathologic histologic manifestations, but all of which he would include in the broad group of rheumatic disease. The first case was one of classic rheumatic polyarthritis, with widespread vascular involvement. The second was a case of less active polyarthritic disease, in which there was marked reaction of the blood vessels of the synovial and joint tissues; the descriptive term applied to this case is chronic polyarthritis vasculosa. The third case was one of obliterating endophlebitis of the liver. The fourth case was one that began with pains in the joints and ended in sepsis; there was marked arteritis, with some lesions that were rheumatic and others that resembled periarteritis nodosa. The fifth case, one of rheumatic periarteritis, Rössle calls tuberculoid periarteritis nodosa. The periarterial granulomatous lesions of the spleen, liver and kidney had led to a gross anatomic diagnosis of miliary tuberculosis. The lesions had the histologic structure of giant Aschoff bodies. Similar smaller lesions were present in the myocardium. From the histologic study of this series of cases Rössle concludes that the limits of what is to be included under rheumatic infection must be broadened to include a wide variety of mesenchymal reactions.

O. T. SCHULTZ.

HEALING STAGES OF PERIARTERITIS NODOSA AND THEIR RELATION TO JUVENILE ATHEROSCLEROSIS. E. JÄGER, Virchows Arch. f. path. Anat. **288**: 833, 1933.

Healed lesions of periarteritis nodosa are rarely seen, because the disease is usually a continuous and progressive process. In three men who died at the ages of 37, 30 and 39, the clinical duration of the illness was thirty-seven, fourteen and five months, respectively. In the first case a clinical diagnosis of periarteritis nodosa had been made early in the course of the disease. In each case necropsy revealed marked atherosclerosis. Microscopic study brought to light the changes in the small arteries which Jäger describes as the healed and healing lesions of periarteritis. In the case of shortest duration active lesions were also present. Injury to the media leads to hyperplasia of the overlying intima in the form of localized nodular thickenings. A single vessel may reveal several intimal nodules of different ages. Injury to the wall during the active stage may lead to the formation of miliary aneurysms; such aneurysms were seen especially in the coronary arteries. In the organization of thrombosed arteries or aneurysms, blood channels are formed; about these there develop a new elastic membrane and a new media. The intimal hyperplasia bears no direct relation to lipoid infiltration, although the intimal nodules may sometimes be formed over what were originally superficial areas of lipoid infiltration. The formation of the intimal callus is not the result of atherosclerosis; when atheromatous degeneration occurs in the callus it is the result of changes in the ground substance. Nodose atherosclerosis in younger persons, when associated with epicardial proliferation over the nodular areas of the coronary arteries and with scars of infarcts in the spleen and kidney, should suggest the possibility of healed periarteritis nodosa.

O. T. SCHULTZ.

CHANGES IN THE NERVES, SKIN AND BONES IN RECKLINGHAUSEN'S NEUROFIBROMATOSIS. A. STALMANN, Virchows Arch. f. path. Anat. **289**:96, 1933.

This investigation is based on the clinical study of thirty-five cases of neurofibromatosis, brief summaries of which are presented. Whenever possible, lesions of the skin, nerves and bones were removed during life and subjected to histologic study. One case came to necropsy. Pigmentary anomalies of the skin are the most common lesions, and are usually of such histologic character that they can be distinguished from other pigmentary nevi. The cutaneous tumors are mesenchymal overgrowths that may arise in connection with the nerves of the skin. Keloids develop in scars as the result of a congenital predisposition of the mesenchyme to overgrowth. The lesions of the peripheral nerves described are the neurinoma and the plexiform neuroma. The lesions of the bones are dystrophic, and are the result of softening followed by deformity. Destruction of bone with pseudarthrosis is frequent in childhood. Psychic disturbances are the result of the participation of the central nervous system in the disease process. Changes in the organs of internal secretion are ascribed to the dystrophic action of the vegetative nervous system. Neurofibromatosis is held by the author to be a blastomatous disease of the nervous system, which has its origin in the embryonic period of life. Abnormal impulses from the diseased nervous system cause functional and anatomic abnormalities of the various organs, the various lesions of which are part of the disease itself.

O. T. SCHULTZ.

HYPERTROPHY OF THE ESOPHAGUS AND STOMACH IN A CASE OF RECKLINGHAUSEN'S NEUROFIBROMATOSIS. H. J. SCHERER, Virchows Arch. f. path. Anat. **289**:127, 1933.

The localized hypertrophy of organs or limbs that is not infrequently associated with neurofibromatosis is held by some to be a concomitant but independent maldevelopment, and by others to be an essential part of the disease due to trophic disturbances through involved nerves. The condition here reported occurred in a man who died at the age of 53 as the result of hemorrhage from an acute ulcer of the cardiac end of the stomach. The external manifestations were those of typical neurofibromatosis. Internal examination at necropsy revealed fusiform and nodular enlargement of both vagus nerves and marked thickening of the wall of the esophagus and stomach. The appendix was abnormally long and thick. Microscopically, the thickening of this organ was due to neurinomatous hyperplasia of the submucosa. Increase in the thickness of the wall of the esophagus and stomach was due to hyperplasia of the circular and longitudinal muscle, without an abnormal increase in the nerve elements. The axis-cylinders of the vagi were separated by neurinomatous proliferation, but were not interrupted or appreciably damaged, although they exhibited slight fusiform enlargements. The author interprets the muscular hypertrophy of the esophagus and stomach as the result of abnormal stimuli that reached these organs through the altered vagus nerves.

O. T. SCHULTZ.

RECURRENT ENDOMETRIOSIS OF LAPAROTOMY SCAR. M. VERSÉ, Virchows Arch. f. path. Anat. **289**:186, 1933.

This is a brief report of an endometrial nodule that developed in a laparotomy scar three fourths of a year after a similar nodule had been excised from the scar. The first nodule was removed one and three-fourths years after laparotomy and an operation on the uterus.

O. T. SCHULTZ.

EPIDERMAL CYSTS FOLLOWING THE INJECTION OF IRRADIATED ERGOSTEROL. H. SANDER, Virchows Arch. f. path. Anat. **289**:190, 1933.

Epidermal cysts developed at the site of injection of irradiated ergosterol into the vein of the ear in thirteen of twenty-two rabbits. The cysts were derived from hair follicles and sebaceous glands. Ergosterol that had not been irradiated did not have a similar effect.

O. T. SCHULTZ.

COMPARISON OF COMPENSATORY HYPERTROPHY OF THE THYROID GLAND WITH THAT FOLLOWING THE ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT. MARTHA SILBERBERG, Virchows Arch. f. path. Anat. **289**:201, 1933.

Previous work had shown that the intraperitoneal injection of an extract of the adenohypophysis leads to hypertrophy of the thyroid gland characterized by a change in the character of the colloid, multiplication of the follicular epithelium and a change in the character of the epithelium. The basal metabolic rate is increased. The compensatory hypertrophy that follows the removal of a lobe is characterized by an increase in the number of epithelial cells, with no change in their character. The growth stimulus of resection of a lobe is therefore held to be purely quantitative in its effect, whereas the stimulus of pituitary extract is qualitative in its effect. The purpose of the present experiments, which were carried out on guinea-pigs, was to determine the influence of the two kinds of growth stimuli when applied simultaneously or one after the other. The experiments consisted of a control series to determine the characteristics of compensatory hypertrophy alone; a series in which resection was followed by the administration of pituitary extract, with variable periods of rest before the thyroid was subjected to examination; a series in which resection followed the administration of the extract, and a series in which resection followed the injection of the extract and was succeeded by another series of injections of extract. In the normal animal the qualitative effects of the injection of pituitary extract reach their maximum after six injections, and the quantitative effects of compensatory hypertrophy reach their maximum at the end of eighteen days. When the extract was administered immediately following resection of a lobe of the thyroid gland, there was no summation of the two effects, but the qualitative effect of the pituitary extract was held in abeyance until the quantitative effect of resection had run its normal course. If the administration of extract was continued for only a short period after resection, the completion of the compensatory hypertrophy was delayed. The maximum degree of change resulted when the compensatory hypertrophy of a thyroid previously stimulated by pituitary extract was allowed to run its course and another series of injections was then given. The quantitative and qualitative growth stimuli interfere with each other, but by the proper alternation of periods of rest and of stimulation the maxima of the quantitative and qualitative effects can be made to coincide, resulting in a thyroid that has the histologic structure of the abnormally functioning hyperplastic thyroid of exophthalmic goiter.

O. T. SCHULTZ.

DWARFISM DUE TO CONGENITAL SYPHILIS OF THE HYPOPHYSIS. RUTH KATZENSTEIN, Virchows Arch. f. Path. Anat. **289**:222, 1933.

A woman, 131 cm. in height, with the stigmas of congenital syphilis, died at the age of 51 of cerebral hemorrhage. Growth had been retarded since the third year of life; she was backward in mental development; she had never menstruated, and since the age of 30 she had had disturbance of locomotion and vision of a progressive character. At necropsy the external and internal genitalia were found to be rudimentary, the ovaries apparently never having functioned. The sella turcica and the hypophysis were small. The hypophysis had a thick fibrous capsule; the posterior lobe appeared normal, but the anterior lobe contained only remnants of glandular tissue in its peripheral portion and these were separated by broad bands of connective tissue. The remnants of the adenohypophysis contained no basophil cells. There were no active inflammatory changes in the pituitary gland; the changes noted were ascribed to congenital syphilis which had run its course. The thyroid gland was small and fibrous. The internal organs were hypoplastic. The author considers the hypophyseal involvement the basic condition. It led to retardation of growth in length, and to atrophy of the thyroid and ovaries. The changes in the latter organs led to failure of development of the genitalia and to hypoplasia of the other internal organs.

O. T. SCHULTZ.

PLACENTAL CHANGES IN SYPHILIS. T. E. OLIN, Arb. a. d. path. Inst. d. Univ. Helsingfors **6**:377, 1931.

These studies are based on thirty-nine cases of syphilitic infection. Microscopic study demonstrated leukocytic infiltration of the decidua basalis at the marginal portion of the placenta. Diffuse infiltration was noted elsewhere in the decidua and in the intervillous septums. Hyperplasia of the villi was observed in the chorion frondosum, as were leukocytic infiltration and abscesses of the villi. The latter occur in two forms: (1) abscesses showing proliferative changes in the stroma with partial destruction of the syncytium (the production of fibrin, overgrowth of collagenous connective tissue and even giant cells may be noted); (2) the exudative type, in which the infiltrated villi fuse and combine with other tissues (macroscopically, the center of the inflamed area may become soft and form abscess-like cavities; edema of the villi is often present). The blood vessels show endothelial proliferation, thickening of the intima and, frequently, obliteration with lymphocytic infiltration. Leukocytic infiltration of the chorion is comparatively frequent. It affects the chorionic cells, the walls of the blood vessels and the stroma. There is usually associated inflammation of the amniotic membrane.

The chief changes in the umbilical cord are in the blood vessels, which show endothelial proliferation, lymphocytic infiltration, even into Wharton's jelly, and destruction of the elastic coat. The veins are more frequently and severely involved than the arteries.

Spirochetes were demonstrated in the placenta in three of the thirty-nine cases.

Antisyphilitic treatment may inhibit to a high degree most of the pathologic reactions. In general, it is concluded that one may be fairly sure that there is syphilitic infection of the placenta when hyperplasia of the villi, miliary abscesses, inflammatory changes in the cord and vascular lesions are present.

J. KLEIN.

Microbiology and Parsitology

GROWTH OF CLOSTRIDIUM BOTULINUM ON SYNTHETIC MEDIUM. W. BURROWS, J. Infect. Dis. **52**:126, 1933.

Evidence supporting the hypothesis of essential amino-acids for bacteria is presented. Certain strains of Clostridium botulinum grew well on certain synthetic mediums. The amino-acids cystine, leucine and proline were essential to the growth of the strains of Cl. botulinum used. The amino-acids lysine and glycine, while not essential, were active in promoting growth of these strains of Cl. botulinum. Isoleucine and oxyproline could be substittued for leucine and proline, respectively, as essential amino-acids. The obligate anaerobes, Clostridium welchii, Clostridium chauvaei, Clostridium sporogenes, Clostridium histolyticum and Clostridium tetani would not grow on a simple sympathetic medium which would support the growth of these strains of Cl. botulinum. The type A culture of Cl. botulinum used showed better growth on these mediums than either of the two type B cultures.

AUTHOR'S SUMMARY.

LITHIUM CHLORIDE MEDIUMS FOR PRESERVATION AND RECOVERY OF THE TYPHOID BACILLUS IN FECES. L. C. HAVENS and C. R. MAYFIELD, J. Infect. Dis. **52**:157, 1933.

Lithium chloride has a sharp selective action on the typhoid-colon group. In mediums containing concentrations of 0.5 per cent, lactose-fermenting organisms are inhibited, while Bacillus typhosus and many of the Salmonella group grow well. The advantages of a simple, inorganic compound with uniform action over a complex substance such as brilliant green, which must be titrated and carefully adjusted, are obvious. Mediums containing lithium chloride, both for preservation of feces and for isolation of typhoid bacilli, have been described. The results of comparative tests indicate that acidified bile containing 0.5 per cent lithium chloride,

and 30 per cent glycerin containing this salt, are superior to other preservative mediums. The addition of 0.5 per cent lithium chloride to Endo agar greatly enhances the chances of recovery of small numbers of typhoid bacilli. There is a sharp decrease in the number of colonies of normal fecal bacteria, with a corresponding increase in the number of typhoid colonies. The motility of B. typhosus on lithium chloride mediums is usually reduced or entirely lost. Agglutination is of the somatic (O) type, in small, granular clumps. The colonies are smaller, more compact and less translucent. All of these characteristics return to the normal on transfer of the organism to ordinary mediums. Repeated platings from specimens of feces over a period of several days result in a significant increase in the number of positive results. This is particularly true when the preservative medium causes a progressive decrease in the fecal flora originally present.

AUTHORS' SUMMARY.

CATAPHORETIC VELOCITY OF STREPTOCOCCI IN ENCEPHALITIS AND OTHER DISEASES OF THE NERVOUS SYSTEM. E. C. ROSENOW and L. B. JENSEN, J. Infect. Dis. **52**:167, 1933.

The results of a study of the cataphoretic velocities of the streptococci isolated from atria of infection of persons suffering from encephalitis and other diseases of the nervous system, and from animals in which characteristic symptoms developed, or which died, following inoculation of the streptococci, are reported. The streptococci as isolated from the nasopharynx and other atria of infection, especially the apexes of pulpless teeth, and sometimes from the blood and involved organs of persons suffering from encephalitis and other diseases of the nervous system, possess a characteristic neurotropic cataphoretic velocity. The velocity of the streptococci as isolated in cases of encephalitis shifted toward the slow velocity of the streptococci from influenza during epidemic waves of influenza. The marked neurotropic type of velocity of the streptococci found during convalescence from influenza suggests, perhaps, why encephalitis and other diseases of the nervous system, such as epidemic hiccup, polio-encephalomyelitis, radiculitis and neuritis, are so prone to occur following attacks of influenza or epidemics of influenza. The serums of almost all patients having encephalitis and certain other diseases of the nervous system had specific velocity-slowing effects on the respective strains of streptococci isolated in cases of encephalitis and having neurotropic velocity. Cataphoretic measurements of the streptococci isolated from atria of infection and measurements of the specific slowing effect of the serum of the patient have proved of value in the differential diagnosis in puzzling cases. Prolonged use of vaccines containing streptococci having chiefly neurotropic velocity, as isolated especially from animals given injections of material derived from patients having encephalitis and other diseases of the nervous system, has been followed by improvement in symptoms and concomitant disappearance of neurotropic streptococci from the nasopharynx in some cases. The results of the studies by the new methods support the hypothesis that streptococci having a common physical characteristic, namely, neurotropic cataphoretic velocity, are directly or indirectly etiologic in the diseases of the nervous system studied.

AUTHORS' SUMMARY.

ANAEROBIC GRAM-NEGATIVE BACILLI FROM ABSCESS OF THE LUNG: TOXIN PRODUCTION AS DEMONSTRATED BY THE SHWARTZMAN PHENOMENON. J. COHEN, J. Infect. Dis. **52**:185, 1933.

In this paper there are reported additional observations concerning isolation, cultivation and classification of certain gram-negative anaerobic bacilli isolated from abscess of the lung. For the study of the toxic factors produced by these bacilli, the Shwartzman phenomenon was employed. When the organisms were grown in the culture mediums described in the text and their filtrates injected intravenously into rabbits which had been prepared twenty-four hours previously by cutaneous injection of potent heterologous toxins (i. e., meningococcus toxin),

approximately 80 per cent of the rabbits showed areas of hemorrhagic necrosis. With some organisms from abscess of the lung, attempts were made to reproduce the Shwartzman phenomenon by the use of homologous filtrates for cutaneous preparation. Of these organisms, only filtrates of Bacterium melaninogenicum (in symbiosis with Streptococcus gamma) and Leptothrix proved potent. Further work on the pathogenicity of the various organisms, to be reported, points to the interesting fact that only those organisms which give the positive Shwartzman phenomenon when they are grown in suitable culture mediums are also able to produce severe necrotizing lesions in the lungs of rabbits.

AUTHOR'S SUMMARY.

SELECTIVE ACTION OF CRYSTAL VIOLET AND OF BRILLIANT GREEN ON BACTERIOPHAGES. A. Y. WELLS and N. P. SHERWOOD, J. Infect. Dis. **52**:209, 1933.

A selective phagistatic action of crystal violet and brilliant green on the bacteriophages tested has been demonstrated, and the results suggest that there is a correlation between the selective action of crystal violet on bacteria and their respective bacteriophages. High concentrations of dyes were required to effect inhibition of the bacteriophages. A high dilution of the primary dye solutions (inhibiting dilutions) to the point where there would be no inhibition of bacterial growth was a requisite in demonstrating the activity or inactivity of the lytic principles after each period of incubation. Under the conditions of the experiment, the bacteriophages that lysed gram-positive bacteria were completely inhibited by concentrations of crystal violet which did not appear to diminish the activity of the bacteriophages that lysed gram-negative bacteria. The inhibitive property of brilliant green was extremely variable when compared with that of crystal violet, but nevertheless distinct with one bacteriophage of the colon bacillus in particular.

AUTHORS' SUMMARY.

ACTINOBACILLOSIS OF CATTLE IN THE UNITED STATES. L. THOMPSON, J. Infect. Dis. **52**:223, 1933.

Actinobacillosis is common among cattle in the United States. It would seem that the condition here is similar to that in other countries in which the greater percentage of the so-called bovine actinomycosis is due to Actinobacillus lignieresi. The condition known as "wooden tongue" is said by European investigators to be entirely due to this organism. One case of this kind in the present study yielded a culture of Actinobacillus. Most cases of involvement only of the cervical glands were due to Actinobacillus. On the other hand, the few cases of infection of bone encountered in this series were due to Actinomyces. Both Actinobacillus and Actinomyces produce the aggregations known as "sulphur granules." The quickest way of distinguishing the two organisms in pus is by staining the crushed granules by Gram's method. Actinomyces granules stained in this way show numerous gram-positive, rod-shaped forms, whereas those of Actinobacillus give no gram-positive organisms, but with careful search a few small, gram-negative, bacillary forms may usually be found. The only points of similarity between the two organisms are the ability to produce similar lesions in cattle and the ability to form sulphur granules. The granules formed by Actinobacillus are usually less conspicuous and can often be found only with the aid of the microscope. Agglutinations and absorptions of agglutinins indicate that there is variation among strains of Actinobacillus as to antigenic structure. There is, however, a certain amount of cross-agglutination in all strains. Considerable experience with various strains and with control serum would be necessary before the agglutination test could be relied on to detect infection among cattle. It is possible that a better way of preparing antigens might be found.

AUTHOR'S SUMMARY.

THE PATHOLOGY OF PSITTACOSIS IN ANIMALS AND THE DISTRIBUTION OF RICKETTSIA PSITTACI IN THE TISSUES OF MAN AND ANIMALS. R. D. LILLIE, Nat. Inst. Health, Bull. 161, 1933.

The lesions of psittacosis in naturally and experimentally infected parrots and parrakeets are described. Rickettsia psittaci has been demonstrated in epithelial cells of the small intestine, ureter, renal secreting tubules and bile ducts, in epithelioid cells, macrophages, reticulo-endothelial cells and mesothelial cells of parrots, in the epithelium of the renal collecting tubules in parrakeets, and in macrophages and alveolar epithelial cells in the lung and in hepatic cells in man. It seems indicated that Rickettsia psittaci is primarily an epithelial parasite, and enters cells of the macrophage-reticulo-endothelial series secondarily as the epithelial cells break down. An etiologic relationship of Rickettsia psittaci to psittacosis seems indicated, though not proved.

THE LIVER IN A FATAL CASE OF EPIDEMIC "CATARRHAL" JAUNDICE. J. F. GASKELL, J. Path. & Bact. **36**:257, 1933.

A case of death from hemorrhage on the third day of acute epidemic "catarrhal" jaundice is described in a patient who had had tonsils and adenoids removed. The condition found was acute hepatitis, the biliary canal system being permeable throughout and intact. The case was not due to spirochetal infection. It is concluded that epidemic "catarrhal" jaundice is a hepatitis. The condition found supports the view that acute and subacute atrophy of the liver are the severe types of epidemic infective jaundice or its sporadic form.

AUTHOR'S SUMMARY.

VARIANTS OF CLOSTRIDIUM WELCHII. C. A. MCGAUGHEY, J. Path. & Bact. **36**: 263, 933.

From an authentic strain of Clostridium welchii, by selection and repeated subculture of colonies, two variants have been isolated which have remained stable during a period of observation of about four years. The variants, designated 1 and 2, differed from the parent strain and from each other in form of colony, growth in broth, the size and shape of individual bacilli, sporulation and formation of capsules. They resembled the parent strain and each other in fermentation activity, in giving an acrolein test, in liquefying gelatin and in producing a stormy clot in milk. Variant 1 at one stage appeared to be incapable of producing toxin but later produced small amounts of toxin in culture. Variant 2 produced from three to six times as much toxin as the parent strain. The production of hemolysin in culture followed exactly changes in the production of toxin. By agglutination tests the antigens of both variants differed completely from the parent strain. The antigens of variants 1 and 2 were closely related and perhaps identical. Mucoid types of the parent strain and of both variants have been noted. The importance of these results in the production of Cl. welchii toxin and their possible application to the study of Cl. welchii infection and immunity is suggested.

AUTHOR'S SUMMARY.

DISSOCIATION IN CERTAIN MYCOBACTERIA. M. H. CHRISTISON, J. Path. & Bact. **36**:285, 1933.

Brinckeroff's "lepra" bacillus, the fish tubercle bacillus (Cobbett), the tortoise tubercle bacillus (Friedmann) and Mycobacterium rubrum (Sohngen 335) dissociate into numerous colony types varying greatly in the complexity of their structure. The colonies show varying degrees of stability which depend to some extent on environmental conditions. There is no evidence of absolute stability among any of the variants, and therefore an attempt to base a classification of these

organisms on colony types and their related characters is regarded as impracticable. Variation also occurs in other characters, such as the type of growth in fluid medium, emulsibility in saline solution, cell morphology, pigment formation and, in the case of the fish tubercle bacillus, ability to absorb dye from gentian violet-egg medium.

AUTHOR'S SUMMARY.

PNEUMOCOCCAL INFECTION IN INFANCY AND CHILDHOOD. J. W. S. BLACKLOCK and K. J. GUTHRIE, J. Path. & Bact. **36**:349, 1933.

The pathology and bacteriology of pneumococcic infections in infants and children of the hospital class are discussed. In cases of acute respiratory disease, throat swabs were used for isolating the pneumococcus as sputum is not easily obtained in the young child. In 38.6 per cent of healthy children, pneumococci were found in the throat, 96.7 per cent of these being group IV strains. The accuracy of the results of the examination of the throat swab in a series of cases of pneumonia were confirmed in 88.5 per cent. Some of the cases of pneumonia which did not come to autopsy could not be definitely classified. In a few cases the lesions in the lungs histologically showed characters of both lobar pneumonia and bronchopneumonia; such mixed types were most frequent in the second year of life. Pneumococci from 140 cases of pneumonia were typed, 9.3 per cent belonging to the fixed types (chiefly types I and II) and 90.7 per cent to group IV. The mortality rate of the fixed type infections was 38.5 per cent and of the group IV infections, 40.9 per cent. In adult cases of lobar pneumonia investigated simultaneously in the same city, 73 per cent were due to types I, II and III pneumococci and only 27 per cent to group IV. The bacteriology of bronchopneumonia was studied post mortem in a series of cases. Pneumococci were found in 85.5 per cent, streptococci in 7.3 per cent, influenza bacilli in 6.1 per cent and Staphylococcus aureus in 1.1 per cent. Pus from the pleura in 114 cases of empyema yielded pneumococci in 68.4 per cent, streptococci in 17.5 per cent and other organisms in 14 per cent. The mortality was slightly higher in pneumococcic than in streptococcic empyemas. Pneumococci were typed in 66 cases of empyema, and 30 belonged to type I, 1 to type II, and 35 to group IV. The incidence of fixed type infections rose with the age of the child, group IV strains predominating in the empyemas following bronchopneumonia in early childhood. The mortality rate in fixed type infections was 32.2 per cent, and in group IV infections, 54.3 per cent. Of 27 cases of primary pneumococcic peritonitis, 24 (88.9 per cent) were in girls and 3 in boys. In 10 of the 11 female children examined at autopsy, the peritoneal lesion was most acute in the pelvis. Of the 3 boys, only 1 was examined post mortem, when no lesion except peritonitis was found. In 13 cases type I, and in 2 cases type II, pneumococci were isolated from the peritoneal exudate. Pneumococcic peritonitis secondary to pneumonia was rare, occurring in only 2.4 per cent of the cases, and in these the thoracic lesions were severe. Pneumococcic meningitis occurred most frequently as a sequel to acute otitis media and was primary in only 17.6 per cent of the cases studied. Of the pneumococci isolated from the cerebrospinal fluid, 84.2 per cent belonged to group IV. Purulent otitis media was present in 21.1 per cent of 2,000 consecutive autopsies; the pneumococcus was the predominating organism in the pus from this lesion. The commonest complication in a series of cases of pneumonia examined post mortem was acute otitis media. In 33 cases of acute middle ear disease, pneumococci were typed, 93.9 per cent being group IV strains. Infection was due to group IV pneumococci in 70.6 per cent of a series of surgical lesions, (arthritis, orchitis and acute abscesses). On arranging the 292 strains of pneumococci isolated from various acute lesions according to type of coccus and age of patient, it was found that the percentage of infections with fixed types was much greater in older children than in those under 3 years.

AUTHORS' SUMMARY.

DIPHTHERIA-LIKE ORGANISMS FROM NASOPHARYNX. M. M. BARRATT, J. Path. & Bact. **36**:369, 1933.

A group of aberrant diphtheria-like organisms have been described which, while resembling Corynebacterium diphtheriae, differ from it in certain cultural characters, most notably in their capacity to liquefy gelatin. The lesions produced in guinea-pigs by intradermal injection of the organisms are more papular and pustular than those following injection of typical C. diphtheriae. Diphtheria antitoxin does not prevent these cutaneous lesions and even in large doses does not save the life of the animal if more than one or two minimal lethal doses are given subcutaneously or with some strains even if only one is given. Toxic filtrates produce lesions of the skin which do not resemble those of C. diphtheriae. Diphtheria antitoxin, unless given in larger doses than from 80 to 100 units to one minimum lethal dose of toxin, does not protect the animal from death. The aberrant strains differ further from C. diphtheriae in being pathogenic for rats by intraperitoneal inoculation. When the characters of the aberrant strains differ culturally or biologically from those of C. diphtheriae, they approach, if not completely, those of Corynebacterium pseudotuberculosis-ovis (bacillus of Preisz-Nocard). Although evidence is lacking of the association of the strains studied with severe cases of clinical diphtheria, the occurrence in the human nasopharynx of diphtheria-like organisms, the pathogenic effects of which in the guinea-pig are not prevented by diphtheria antitoxin, has been established. Therefore, the possibility that strains of this type play a part in the causation of cases which do not respond favorably to antitoxin cannot be altogether ignored. AUTHOR'S SUMMARY.

QUANTITATIVE DETERMINATION OF BACTERIOPHAGE ACTIVITY. G. DREYER and M. L. CAMPBELL-RENTON, J. Path. & Bact. **36**:399, 1933.

Evidence has been produced to show that the generally accepted view that the number of plaques produced increases in direct proportion to the increase in the concentration of the bacteriophage does not hold, but that a relatively smaller number of plaques is produced by the higher concentration. The ratio between the concentration of the bacteriophage and the number of plaques formed may be represented graphically by a "standard" curve, based on our observations. This standard curve is applicable to all the experiments we have carried out, independent of the strength of the bacteriophage, the number of bacteria on which it acts and the concentration of agar. It also seems to apply to results obtained by some previous workers. This standard curve constitutes a basis and fixed standard for the quantitative determination of the activity of any bacteriophage. We have shown, in agreement with Gratia, Gjörup and Burnet, and contrary to the observations of d'Herelle, Bronfenbrenner and Korb, Asheshov, Marshall and others, that the number of plaques produced by the same concentration of bacteriophage increases with increasing numbers of bacteria, up to a maximum, and then tends to decrease; at the same time the size of the plaques is diminished. When bacteriophage acts on bacteria spread on 1.5 and 4 per cent agar, respectively, fewer and smaller plaques are produced by the same concentration of bacteriophage on the strong agar than on the weak agar. The admixture of homologous organisms killed at 100 C. to the inoculum reduces the activity of the bacteriophage. It has been found that the Pasteur bacteriophage used for our experiments deteriorates rapidly at 37 C., and that the rate of deterioration may be satisfactorily expressed by the equation for a monomolecular reaction. AUTHORS' SUMMARY.

THE APPENDIX IN THE PRODROMAL STAGE OF MEASLES. W. H. SCHULTZE, München. med. Wchnschr. **80**:576, 1933.

Histologic sections of an appendix removed from a girl 10 years of age, with appendical symptoms, showed no evidence of acute appendicitis, but did show large numbers of giant cells in the mucosa and submucosa. Ten or more nuclei were present in the cells. Schultze was familiar with the findings of similar giant cells

in the appendix during the prodromal stage of measles which were reported by Finkeldey (Finkeldey, W.: *Virchows Arch. f. path. Anat.* **284**:518, 1932) and by Walter Fischer at Wiesbaden in 1932. Schultze diagnosed measles from the histologic picture, and a typical rash actually developed in the child on the day following the operation. Schultze was apparently unaware of the fact that the first case of this type was reported in the American literature by Herzberg in January, 1932 (*J. A. M. A.* **98**:139, 1932).

I. DAVIDSOHN.

Immunology

TUBERCULOUS SKIN REACTION. E. S. MARIETTE and E. P. K. FENGER, Am. Rev. Tuberc. **25**:357, 1932.

The M A 100 human protein (identical with the original tuberculoprotein isolated by Seibert) is as sensitive and as selective as old tuberculin, and probably more so. The initial and subsequent doses recommended for the M A 100 human protein are safe, in that dangerous reactions are not encountered. The initial and subsequent doses recommended for M A 100 human protein are large enough to identify the majority of tuberculous persons, and apparently do not need to be increased. The M A 100 proteins are apparently not specific, at least in large doses. There is apparently a protein substance common to all acid-fast bacilli, which if given in large enough doses, will elicit the same type of reaction as that obtained from old tuberculin. As the M A 100 protein represents a substance in a purified form which can always be reproduced at the same iso-electric point and which can be weighed out in milligram doses, so that the exact content of the solution in milligrams is known, it is a better testing substance than old tuberculin.

H. J. CORPER.

SERUM SICKNESS IN RABBITS. M. S. FLEISHER and L. JONES, J. Immunol. **24**:369 and 383, 1933.

The serum of individual normal horses shows variations in activity in producing serum sickness in rabbits, so that the occurrence of this manifestation may vary between 93 and 24 per cent. Similar variations in the activity of two batches of pooled serums have been noted, the variations being between 60 and 35 per cent. Differences have also been noted in the activity of antiserums of various types. No evidence has been obtained suggesting that the immunization of animals increases or materially alters in any way the possibility of the serum causing serum sickness. It appears that beef serum is probably less active in causing serum sickness than is horse serum; and sheep or hog serum is definitely less active than horse or beef serum in this regard. The addition of as much as a 0.35 per cent concentration of phenol to serum does not apparently alter its activity in causing serum sickness. Aging of the serum (up to nineteen months) does not alter its power to cause serum sickness. The reactivity of the individual animal may be a determining factor in the occurrence of serum sickness.

When rabbits receive a second injection of serum after an interval of time, they may show reactions of immediate, accelerated or normal serum sickness. These reactions in the rabbits are essentially analogous to the reactions appearing in man subsequent to a second injection of serum. The time elapsing between the first and second injections appears to be a factor in determining the type of reaction which will occur. If only twelve days elapse, no abnormal reactions are noted; if about two weeks, a few mild accelerated reactions appear; and if from twenty to thirty-three days, a larger number of more marked accelerated reactions are evident, and a few immediate reactions occur. At from thirty-six to fifty-three days more immediate reactions and quite a number of accelerated reactions occur; at this period abnormal reactions occur in a larger percentage of animals than at any other. At from three to four and one-half months only immediate reactions were observed. Finally, at six months no immediate reactions were produced; a few accelerated reactions were noted, and a number of reactions which were on the borderline between accelerated and normal and a number of normal reactions were observed.

AUTHORS' SUMMARIES.

HEMORRHAGIC ALLERGY: II. SHWARTZMAN AND ARTHUS PHENOMENA. ANDRÉ GRATIA and ROGER LINZ, Ann. Inst. Pasteur **50**:89, 1933.

In rabbits given 0.5 cc. of horse serum intravenously followed after eight weeks by 0.1 cc. injected intradermally the edema of the Arthus phenomenon developed. As the test was repeated more congestion and less edema were apparent, and the lesion finally appeared as an intense hemorrhagic lesion identical with Shwartzman's reaction and not given in the classic descriptions of the Arthus phenomenon. From about 15 to 20 per cent of the experiments performed on rabbits yielded in a marked degree the result described, and as many animals were refractory—a distribution correlating with the Shwartzman reaction, and including identical animals. Fatal anaphylactic shock occurred in the animals with the hemorrhagic lesions, but not in the refractory animals. Both the Arthus and Shwartzman reactions occurred in the same animal simultaneously, appearing eventually identical and without reciprocal desensitization. Each reaction, however, failed to desensitize specifically against its own type. Both reactions desensitized guinea-pigs and rabbits against serum shocks, often only partially. Reciprocally, a nonfatal serum shock desensitized these animals against the Arthus reaction but apparently not against the more violent Shwartzman reaction. There appears to be only a simple quantitative difference in intensity and rapidity between these reactions, as though microbial products stimulated a local hypersensitivity in a few hours, whereas other antigens required several weeks. FROM AUTHORS' CONCLUSIONS.

GRUNDFRAGEN DER IMMUNBIOLOGIE UND ALLERGIELEHRE.

This is a special number of *Immunität, Allergie und Infektionskrankheiten*. The editor-in-chief is Fr. Michelsson, and among the associate editors are Degkwitz, Schlossberger, van Leeuwen and others. The publisher is Otto Gmelin, Munich. The journal serves the needs of the practicing physician; its purpose is therefore informatory. The contents are reviews by authors who are recognized authorities on their assigned subjects. In the special issue which is reviewed nine authors discuss on 112 pages subjects well deserving to be summarized, as is done in the titles. The original contributions of the authors form the background against which the results obtained by other authors are projected and discussed. L. Bogendoerfer discusses the relation between immunity and the reticulo-endothelial and endocrine systems. In addition to others, his own experiments have established such relations, as is shown by the fact that severing the spinal cord at the level of the sixth cervical vertebra inhibited the development of agglutinins in dogs. The same inhibition was brought about by the administration of a sympathicotropic drug (ergotamine). E. F. Mueller writes about the skin, the sympathetic nervous system and susceptibility to disease. Ivanic and Dimitrijevic-Speth summarize the biokinetic theory of infection, the experimental basis of which is largely their own contribution. The motility of bacteria is linked with their pathogenicity and virulence in a very convincing manner. A new classification of infectious diseases is deduced. Chapters on latent infection, by Schlossberger and Koch, and on paradental focal infection, by Leschke, follow. Pockels reviews his successful attempts at increasing immunologic responses by the administration of extracts of the spleen and the lungs and of reticular endothelium. Niekerk reviews the present knowledge of allergens and brings out the weaknesses of the hypothesis of the all-important significance of heredity in allergic susceptibility. It is possible that the differences between allergic and normal persons are merely quantitative. Recent investigations on the chemistry of allergens are analyzed. A great deal of information is condensed in 13 pages on allergic diathesis by Kaemmerer. He defines diathesis as an individual, congenital and, frequently, hereditary condition, in which physiologic stimuli produce an abnormal reaction. He advocates the use of the term "diathesis" in preference to the terms "constitution" and "disposition" because the former has the meaning of a nonspecific state and the latter of a transient state. Kaemmerer agrees with the great number of those who are unable to see an absolute difference between anaphylactic conditions in animals and the phenomena of allergic hypersensitivity in man. In the final chapter Hofbauer stresses the significance in asthma of local conditions in the upper respiratory

tract, particularly of the nose, and supports his contentions by means of well selected experiments on animals. In addition to the local conditions, internal secretory disturbances are pathogenic factors.

I. DAVIDSOHN.

DEFENSE MECHANISMS IN CHICKEN SPIROCHETOSIS. I. L. KRITSCHEWSKI and P. L. RUBINSTEIN, Virchows Arch. f. path. Anat. **287**:566, 1932.

Chickens infected with Spirochaeta gallinarum were killed at different intervals. Histologic examination of the organs with special spirochetal stains revealed that the spirochetes gradually become dissolved after undergoing degenerative changes such as splitting and fragmentation. Phagocytic processes were seen only rarely and apparently do not play an essential rôle in the destruction of the spirochetes. It seems most likely, therefore, that the destruction of the spirochetes is due to the action of specific lysins.

W. SAPHIR.

ISOLATION OF GROUP-SPECIFIC AGGLUTINOGENS OF ERYTHROCYTES. A. JUHÁSZ-SCHÄFFER and A. VANNOTH, Ztschr. f. d. ges. exper. Med. **86**:809, 1933.

The authors have attempted to separate the agglutinin factor from the stroma of erythrocytes. This problem is of practical importance for the determination of questionable blood groups. Previous authors (Hektoen, Schulhof and Jacoby) have shown that this factor is of albuminous nature. The agglutinogen is bound to the stroma of the erythrocyte and cannot be demonstrated in the serum. The stroma forms agglutinin even after removal of the hemoglobin. The authors were unable to demonstrate an agglutinogen by washing erythrocytes in a physiologic solution of sodium chloride or by causing hemolysis. Nor were they able to inhibit agglutination by these means. It is concluded that the agglutinin principle of the erythrocytes cannot be separated from the stroma. It seems that iso-agglutination is due to a physicochemical property of the stroma rather than to a special substance.

L. KLEIN.

Tumors

PRODUCTION OF SARCOMA BY BACILLI. V. FISCHL and E. KUSSAT, Ztschr. f. Krebsforsch. **36**:276, 1932.

The experimental results reported earlier in 1932 by Bellows and Askanazy, to the effect that spore-forming bacilli isolated from ripe tomatoes would induce peritoneal sarcomatosis of rats if injected repeatedly, could not be confirmed by these writers. Organisms from other vegetable sources were similarly tested without result.

H. E. EGGERS.

FREQUENCY OF PULMONARY CANCER. A. E. SITSEN, Ztschr. f. Krebsforsch. **36**:313, 1932.

Sitsen questions the authenticity of the statistics that have appeared with some frequency tending to show an increase of cancer of the lung in recent years. He points out that with them attention is seldom paid to the altered age distribution in the general population, and he states that no increase in the incidence of this cancer could be found in statistics collected by him at Innsbruck, after this factor had been evaluated.

H. E. EGGERS.

MALIGNANT TUMORS AND ARTERIOSCLEROSIS. J. CASPER, Ztschr. f. Krebsforsch. **36**:354, 1932.

Casper presents statistical evidence from his own collection that as a rule cancer and arteriosclerosis do not occur in association, though, of course, the rule is one with numerous exceptions. This negative relationship can scarcely be a matter of direct causal association, and Casper suggests that the explanation may be a matter of cholesterol metabolism. Animal experimentation, however, would

tend to show that an excess of cholesterol contributes toward both arteriosclerosis and the occurrence of cancer, so that the explanation, if along these lines, must rest on some as yet unrecognized alteration of cholesterol metabolism.

H. E. Eggers.

Increased Incidence of Pulmonary Cancer. A. Syrek, Ztschr. f. Krebsforsch. **36**:409, 1932.

In autopsy statistics collected by the Gesellschaft der Ärzte at Warsaw, there has been a threefold increase in cancer of the lung from 1925 to 1930 over the corresponding period from 1919 to 1924. As usual, the cases are predominantly in male patients. The Warsaw statistics are of some especial interest, as they apparently rule out certain suggested etiologic factors. Industrial irritation may apparently be excluded, as the cases were largely of rural origin; so, too, the possible effect of automobile exhaust gases. The predominance in males would cast doubt on the effect of previous influenza. Of other suggested factors, the effects of tobacco and of exposure to war gases remain as possibilities.

H. E. Eggers.

Carcinosarcoma. M. Bösenberg, Ztschr. f. Krebsforsch. **36**:416, 1932.

Bösenberg reports six cases of what he considers mixed malignant growths. The first is a squamous cell carcinoma in association with a spindle cell sarcoma; the second, an adenocarcinoma and a mixed cell sarcoma of the liver; the third, a keratinizing prickle cell carcinoma and a mixed cell sarcoma; the fourth, a similar carcinoma with a lymphosarcoma (?) of the uterus; the fifth, a slightly keratinized prickle cell carcinoma and a mixed cell sarcoma of the lung, and the sixth, a basal cell carcinoma and a spindle cell sarcoma of the skin. The author considers the last two cases as collision tumors. A rather extensive bibliography is appended.

H. E. Eggers.

Frequency of Bronchial and Pulmonary Cancers in 1925-1931. E. Disemann, Ztschr. f. Krebsforsch. **36**:563, 1932.

While the yearly incidence of pulmonary cancer as observed at the Deutsche pathologische Institut at Prague during the years considered showed a marked fluctuation at the end of the period the deaths from these cancers were found to constitute 16.47 per cent of all deaths from carcinoma. Eighty of 7,855 autopsies, or 1.02 per cent, revealed bronchial or pulmonary cancers; that is, these types constituted 10.85 per cent of all cases of cancer. This was a definite increase over the preceding five years. In regard to the distribution according to age, the highest incidence was seen in the sixth decade. The increase involved principally the male sex, in which the rate was quadruple that of the preceding five years, while in female patients it was double. A considerable proportion of the patients had been unduly exposed to excessive inhalation of smoke or dust. More than 50 per cent came to Prague from western Bohemia.

H. E. Eggers.

Cytodiagnosis of Malignancy in Puncture Fluids and Secretions. H. Karp, Ztschr. f. Krebsforsch. **36**:579, 1932.

Karp has made a critical study of the method of cytodiagnosis of malignant tumors as suggested by Quensel, and emphasizes the following differential criteria between cells desquamated from serous linings and those of cancerous growth: The former cells are ordinarily from 15 to 20 microns in diameter, are regularly round or ovoid in outline, and frequently are grouped in irregular plaques, the margins of which are obscured by a sheath of structureless proteid material. Fat deposits or vacuoles are present in the cytoplasm with some frequency; the nuclei range from 5 to 12 microns in diameter, and the nucleoli, from 1 to 2 microns. The ratio of nucleolar to nuclear surface varies from 1:25 to 1:100.

With tumor cells, there is much greater variation of size; vacuolation may be characteristic, with the appearance of peculiar giant vacuoles from 40 or 50 microns in diameter to a maximum of 90 microns. Nuclear diameters range from 5 to 24 microns; those of the nucleoli, from 4 to 12 microns. The relative increase of the nucleolus is almost characteristic, and the ratio of areas may range from 1:20 to 1:4. Irregular deposition of chromatin—pyknosis or local accumulation—is more frequent in tumor cells, and hyperchromatosis of the nuclear margin is frequently present. Either type of cell may show phagocytosis. With these criteria, diagnoses have been confirmed at autopsy with some regularity.

H. E. Eggers.

Primary Malignant Tumors of Serous Linings. B. Fischer-Wasels, Ztschr. f. Krebsforsch. **37**:21, 1932.

In the opinion of Fischer-Wasels, the existence of tumors, either benign or malignant, of primary origin in the cells lining the serous body cavities is exceedingly doubtful. Definite demonstration of such origin has still to be made. In tumors which have up to the present been reported as of this character, nothing convincing has been observed. In general, their properties are those of other common tumor forms, usually those of undifferentiated carcinomas. Tumors of unquestionable primary location in these sites are regarded as originating from the results of embryologic displacement. In the author's opinion, the identification of this group of tumors by the demonstration of histologically intermediate cell types is unconvincing and productive of error.

H. E. Eggers.

Increase of Pulmonary Cancer and Its Pathogenesis. W. Nowicki, Ztschr. f. Krebsforsch. **37**:83, 1932.

This study of the relative incidence of pulmonary cancer is based on autopsy material observed at Lwow (Poland) during the last thirty-five years, amounting to approximately 31,000 cases. Of the seven five year periods considered, in the first the incidence of cancer of the lung amounted to 0.07 per cent. From 1916 to 1920, it constituted 0.60 per cent; from 1921 to 1925, 0.38 per cent, and from 1926 to 1930, 0.47 per cent. The corresponding figures as related to total cases of cancer are 1.8, 9.3, 5.3 and 7.2 per cent. The increase is most striking when the last fifteen year period is compared with the similar one preceding; this shows more than doubling of the incidence. Three fourths of the cases occurred in male patients; Jews and Gentiles were affected alike; more than 50 per cent of the cases occurred in persons between the ages of 40 and 60. Tuberculosis was associated only rarely. No definite etiologic factor could be recognized, and Nowicki conjectures that chronic bronchial irritation, by the production of metaplastic changes of the bronchial epithelium and the tendency of such changes to become cancerous, may be of indirect importance.

H. E. Eggers.

Diagnosis of Cancer by Photometric Determinations in Venous Blood. E. Sehrt, Ztschr. f. Krebsforsch. **37**:94, 1932.

Basing his work on the theory that cancer is associated with general and fundamental changes in body oxidation, with involvement of the hemoglobin, Sehrt describes the diagnostic reactions based on the altered behavior of the hemoglobin with respect to oxidative changes. In the first reaction reported, the differential behavior of venous blood in response to α-naphthol and dimethylparaphenylendiamine, with and without the addition of potassium ferricyanide, is noted. The blue colors developed under these circumstances are compared; in normal blood, the difference between them is approximately twice that shown in the presence of cancer. In the second reaction, the basis is the behavior of the hemoglobin in response to reduced methylene blue (methylthionine chloride, U. S. P.); in cases of cancer there is a definite increase of the oxidizing power of the hemoglobin. Sehrt believes that the increased and relatively stable oxygen-combining power of the hemoglobin is at least in part responsible for diminished tissue oxidation, and

he regards this as an important contributing factor in the genesis of cancer. In general, his results were well substantiated by the later course of the cases studied; however, among his normal controls, 12 per cent gave reactions of the cancerous type to his first test, and he regards this as evidence of a cancerous predisposition in these cases. Persons with esophageal cancers gave negative reactions to both tests.

H. E. Eggers.

Cancerigenic Principle of Oils and Fats. T. Gassmann, Ztschr. f. Krebsforsch. **37**:117, 1932.

On the analysis of extracts of crude coal tar and tobacco ash, Gassmann found a phosphorus-iron compound to which he assigned the formula $Fe\ (P_2O)_2$. He believes that this is the active cancerigenic agent of these substances. He found it, in combination with formic acid, in the urine of patients with cancer.

H. E. Eggers.

Cancer of the Female Genitalia in Children. K. K. Ortmann, Ztschr. f. Krebsforsch. **37**:283, 1932.

The author reports a case of vaginal cancer in a child 1½ years of age. Morphologically, the cancer was of an undifferentiated type; the photomicrographs suggest a similarity to the less differentiated squamous cell carcinomas, but it is stated that there were occasional suggestions of tubule formation. Two other cases of vaginal cancer and six of uterine carcinoma occurring in children are cited.

H. E. Eggers.

Atypical Epithelium and Malignant New Growths. H. T. Deelman, Ztschr. f. Krebsforsch. **37**:374, 1932.

Deelman presents a critical study of a number of non-neoplastic atypical epithelial new growths, with the considerations that led to their diagnoses. In the last case, he is forced to acknowledge that the history was an absolutely essential item in the diagnosis. This case was one of syphilitic hypertrophy of the skin. It illustrates strikingly the close resemblance of some of these lesions to cancer of the skin, a resemblance which has worried numerous pathologists.

H. E. Eggers.

Relations Between Benign and Malignant New Growths. H. T. Deelman, Ztschr. f. Krebsforsch. **37**:383, 1932.

The study of experimental tar cancers has shown that with them there are stages in which the character of the lesion as regards malignancy cannot be determined on morphologic evidence. Similarly, there are human lesions which likewise are of uncertain character, and which, being biologically malignant, may fail to betray the fact in their morphology. The so-called transition from a benign to a malignant lesion, in Deelman's opinion, really signifies that morphologic evidence of malignancy has not yet appeared in lesions which are already biologically cancerous.

H. E. Eggers.

Calcified Epithelioma of the Skin. I. Tanasescu and N. P. Balan, Ztschr. f. Krebsforsch. **37**:398, 1932.

The case reported occurred in a child of 8, who had double lesions of apparently infiltrative overgrowth of the skin, with extensive calcification of the more deeply seated epithelial colonies. The infiltration was not malignant in the clinical sense. The authors explain the recurrence after operation as the result of unremoved accessory nodules. A review of similar cases is included.

H. E. Eggers.

INSULAR CARCINOMA OF THE PANCREAS. H. HAMDI, Ztschr. f. Krebsforsch. **37**: 411, 1932.

In the case reported the primary tumor was seated in the tail of the pancreas, with free infiltration throughout the remainder of the organ and numerous metastases in the liver. The diagnosis was made on morphologic grounds alone, and no mention is made of the aberration of sugar metabolism which is usually such a prominent feature of carcinoma of the islands of Langerhans.

H. E. EGGERS.

MALIGNANT TUMORS PRODUCED BY TOMATO JUICE. M. PLONSKIER, Ztschr. f. Krebsforsch. **37**:492, 1932.

Plonskier's experimental observations constitute one of the few exceptions to the negative results obtained by almost all investigators who have attempted to reproduce Bellow's reported induction of sarcoma in rats by the injection of tomato juice. In two of six rats given five injections of tomato juice he observed the appearance of sarcoma, a diagnosis which was confirmed not only by the morphology of these tumors, but also by the presence of extensive metastases in the spleen, liver and lymph glands. However, the tumors were not transferable by inoculation.

H. E. EGGERS.

EXPERIMENTAL PRODUCTION OF SARCOMA IN RATS. G. KLEIN, J. KLINKE and R. HANSER, Ztschr. f. Krebsforsch. **37**:539, 1932.

These writers have attempted unsuccessfully to repeat Bellow's experiments on the induction of sarcoma in rats by the injection of tomato juice or of organisms obtained from it. When intact, not overripe tomatoes were used, bacterial infection appeared only when the skin had been damaged. With fruit of this character, among other organisms bacteria resembling Bacillus subtilis were recovered. But the intraperitoneal injection of these, as well as of filtrates and ultrafiltrates of tomato juice, failed to cause other than granulomatous growths; similar growths were obtained by the injection of the juices of apples and grapes, and their appearance suggested an origin in mechanical irritation. Their morphology, however, suggested the possibility of later sarcomatous change.

H. E. EGGERS.

CANCER OF THE LUNG. W. PETERS, Ztschr. f. Krebsforsch. **37**:587, 1932.

Peters studied the incidence of cancer of the lung from material gathered at the Moabite Krankenhaus in Berlin. The increase has been definite, from the fifth most frequent form of cancer, which was its status in 1905-1906, to the second most frequent in 1925-1926. The increase was principally in the male sex; in 1905-1906 to sex ratio was 3:1, and 1925-1926, 6:1. Cancer of other organs does not show a corresponding increase, and gastric cancer has even diminished in frequency. Statistics from other sources, compiled and compared similarly, as a rule show a similar increase, but there are exceptions, occasionally found in only one of two nearby communities, a phenomenon without explanation. Four cases of cancer of the lung are described; one of them originated in the wall of a tuberculous cavity.

H. E. EGGERS.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Oct. 9, 1933

E. H. HATTON, *President, in the Chair*

THE ORIGIN AND PATHOLOGIC SIGNIFICANCE OF THE EPITHELIUM FOUND ABOUT THE ROOTS OF TEETH: PRESIDENTIAL ADDRESS. E. H. HATTON.

Malassez (Arch. de physiol. norm. et path. **5**:129, 1885) is generally credited with the first description of the epithelial bodies found about the roots of teeth:

"The development of pure epithelial tumors within the jaw, which are remote from known epithelial structures; the resemblance of certain parts of these tumors to different types of epithelium which are concerned in the formation of teeth or which accompany it, and the existence of small cellular bodies in the normal gums of the adult that likewise resemble the same have long since brought me to the belief that in the jaws of adults there must be found epithelial rests originating from the formation of teeth.

"This hypothesis, which is so attractive and probable in its conception demands anatomic confirmation. The result of my work has been the disclosure of small cell nests about the roots of the teeth of adults, and in normal relationships, masses which must be considered as epithelial rests of the tooth-forming process and as the origin of certain intramaxillary new growths."

Malassez examined one lower jaw by histologic methods to supply the evidence on which this report was based. The studies were made on two incisors, one cuspid, two premolars and one molar. He found in most of the sections that: "in truth, small cellular masses existed, which had their location for the most part in the innermost part of the alveolodental ligament close to the substance of the tooth, and, indeed, often almost in contact with the cementum. However, I have found them, less seldom, in the outermost portion adjacent to the bone and, in isolated instances, even in medullary spaces within the contiguous bone. . . . The deepest are found about the extremity of the root; the most superficial are located just beneath the gingival attachment to the tooth. It is these superficial portions that I had previously observed in the gums and that Serres had considered as dental calculus glands.

"Is the presence of these epithelial rests constant, frequent or exceptional? I am not able to answer this question exactly; all that I can say is that I have found them about all the teeth that I have examined. . . . What name shall be given to these epithelial masses, which, so far as I know, have not been described? Because of their origin and nature and in accordance with present terminology, I propose the name *amas epithélieux parodontoire* (periodontal epithelial débris)."

In succeeding sections of the monograph based on these studies and in other monographs Malassez attempted not only to defend his conception of the origin of the periodontal epithelial débris but also to relate it to twelve conditions, among which are: radicular dental fungosities, radicular dental cysts, intramaxillary epithelial tumors, multilocular cysts of the lower jaw, odontoblastic cysts, odontomas and carcinomas. The suspicion remains that he arrived at his conclusions largely by deductive processes rather than by carefully controlled experimentation and extensive laboratory study. On the other hand, his descriptions and illustrations of the material studied do not deviate in any material respect from those of the most recent investigators.

A great deal of unnecessary confusion has arisen concerning the significance of the work of Malassez, because a French anatomist, Serres (Essai sur l'anatomie et la physiologie des dents, ou nouvelle théorie de la dentition, Paris, Mequignon-Marvis, 1817) described epithelial structures in the gums under the name of tartar glands, which are considered by many to be identical with the epithelial débris of Malassez. Malassez, however, was familiar with the work of Serres and insisted that his epithelial débris was entirely distinct in origin and significance from the structures studies by Serres, which are described in the following words:

"The dental glands, because of their minuteness, have hitherto eluded the researches of anatomists. Nevertheless, the gums of the fetus at term contain a considerable quantity. Their function at this time appears to be the lubrication of the cartilages which serve for suction when retaining the nipple of the mother.

"I was led to the discovery of these small glandular bodies in looking for the opening of the gubernaculum dentis on the jaws of a fetus at term. I found four or five whitish bodies side by side; when they were pressed firmly, after a small opening had been made, a white substance of the consistency of wax and assuming a spiral form exuded from them. On examining the two jaws attentively, I found a multitude of similar glands distributed throughout the cartilaginous substance which at this time forms the gums. . . . I detached many of these bodies, the size of which equalled that of a millet seed and which were similar to the meibomian glands. I could not discover any distinct opening; the microscope showed only a small brown point in the middle; the white substance contained in the interior did not exude unless an opening was made in the little sac. These glands, then, appear to be formed of a little sac or cyst secreting and containing this white matter and allowing it to transude by their pores or by the little black point which the microscope revealed. The largest are situated on the inner side of the gums in the kind of groove found in this situation. These glands, as we have previously stated, serve to lubricate the cartilages which in the fetus take the place of the teeth before their appearance, but after the eruption of the teeth they secrete the substance which is known as tartar."

It must be clear from this description that the structures described by Serres are very superficial and have nothing to do with the dental follicle. Further discussion of their character and significance is irrelevant here.

Malassez' contention that his epithelial débris originates from the disintegration of the epithelial structures of the tooth germ was challenged at home and abroad. Magitot and LeGros (J. l'anat. et physiol. **15**:286, 1879) asserted that as soon as the function of the enamel organ had been accomplished it must atrophy and disappear. Von Kölliker (Handbuch der Gewebelehre des Menschen, Leipzig, Wilhelm Engelmann, 1867, p. 494) believed the same. Others, though granting the existence of the epithelial débris, argued that it had nothing to do with the origin of dental root cysts and other pathologic entities listed by Malassez. Of these, perhaps Grawitz is the best known, although his report appeared much later and is reserved for later mention.

Dependable observations accumulated slowly as embryologists and histologists gradually untangled the process of tooth development. Von Brunn (Arch. f. mikr. Anat. **29**:367, 1887) supplied an excellent description of the enamel organ and emphasized that the epithelium not only covers the enamel cap but also extends the full length of the root, enclosing it in a sheath. He insisted that the function of the enamel organ is to give form to the tooth, and that the formation of enamel is merely an accessory function. He said: "Its presence is evidently necessary for the odontoblast being properly placed, namely, against its inner surface; it forms the matrix for the later dentine substance. When the odontoblasts occupy the proper position, when the first layer of dentine has been formed and in this way the shape of the tooth has been secured, the epithelium of the enamel has played its part for that portion of the tooth." Hertwig (Die Elemente der Entwicklungslehre des Menschen und der Wirbelthiere, Jena, Gustav Fischer, 1900) made similar observations, and his name has been attached to the epithelial sheath that surrounds the developing tooth. (An excellent discussion

of this subject has been supplied by Dr. Kaethe W. Dewey [in Moorehead, F. B., and Dewey, K. W.: Pathology of the Mouth, Philadelphia, W. B. Saunders Company, 1925, p. 432].)

It has been assumed that this sheath remains intact until the connective tissue invades it in order to produce the cementum covering of the root, and that at this time its disintegration and atrophy are associated with the formation of the epithelial débris of the periodontal membrane. In this conception the enamel portion of the tooth was considered relatively fixed, and the root with its attendant sheath was thought to grow inward. This classic conception has been challenged by at least one school of histologists, who stated that Hertwig's sheath does not grow inward but that the loop constitutes a relatively fixed point from which the root portion grows outward, carrying the crown with it. Even during the developmental period the sheath is continuous only at the loop portion near the apex, and as the connective tissue portions of the tooth and follicle grow faster than the epithelium, disintegration begins early. However, the sheath plays an important part in the development of the bifurcation of the multirooted teeth, and larger masses of epithelium are deposited at these bifurcations (Orbán, B.: Dental Histology and Embryology, ed. 2, New York, P. Blakiston's Son & Co., 1929, p. 129). This may have some pathologic significance, as will be shown later.

The existence of these epithelial bodies in the periodontal membrane, substantially as described by Malassez, is generally accepted. They are more common about the teeth of young persons and are relatively less common or even rare in older persons. They may be found in the adjacent bone, especially in that of the apical region, and not infrequently in pulp canals of the teeth.

In addition to the epithelium of Hertwig's sheath, that is, the layer adjacent to the developing root, there are other sources of epithelium associated with pathologic processes in this region, namely, the outer epithelial layer of the enamel organ, the inner layer adjacent to the enamel cap, the epithelial extension (cord or lamina) from the mucosa to the dental follicle and the epithelium of the mucous membrane.

On the basis of the foregoing conclusions, the genesis of a dental root cyst may be explained on the assumption that under the stimulation of the infectious process at the apex the epithelial rests in the immediate vicinity increase in size and proliferate into the granulation tissue. At a central position degeneration and liquefaction take place, and in time this cavity has a wall that is continuously lined by this epithelium, except for a defect into which in many cases the apex of the tooth may be inserted. Even in such cases the epithelial lining of the cavity is snugly attached like a collar to the denuded surface of the root. Grawitz (Die epithelführenden Zysten der Zahnwurzeln, Greiswald, J. Abel, 1906) had little regard for this theory, and insisted that the epithelial lining of the wall of the cyst was always derived from the mouth by extension along a fiistulous tract which at one time had connected the infected region with the mouth cavity. In his opinion, a closed cyst arises from the obliteration of the channel from the mouth after the cavity of the cyst has been lined with epithelium. He believed that only true dentigerous cysts can be derived from the epithelium of the tooth embryo. It is difficult, although not impossible, to find supporters of Grawitz' contention today. James and Counsell (Brit. Dent. J. **2**:463, 1932) stated that there is no reason to believe that the epithelial rests play any part in the production of pathologic epithelium.

Most writers, however, would agree with the opinion expressed by Siegmund and Weber (Pathologische Histologie der Mundhöhle, Leipzig, S. Hirzel, 1926, p. 184), that the epithelium which is found not only in dental cysts but also frequently in granulomas has its origin in the epithelial rests of the Hertwig sheath, which are to be found in the periodontal membrane of every tooth. They said: "In not one case in our abundant material have we been able to demonstrate any connection in the sense of Grawitz between mouth epithelium and that of a granuloma through a fistulous tract."

I have seen two such tissues, but in these it was difficult to decide whether epithelium from the mouth had proliferated into the granuloma, or vice versa.

In one it seemed that the epithelium from the apex of the tooth had grown out toward the mucosa. The fact that cysts of the roots of teeth in the upper jaw are occasionally lined with columnar, ciliated epithelium also suggests that in some instances the lining of the cyst may be formed by the proliferation of epithelium from outside sources instead of from epithelial rests.

Since epithelial rests are found in pulp canals, and because of the peculiar relationship of the cells of inner enamel epithelium to the production of dentine, the close association of these rests and denticles is a matter of no little interest. It can be shown that either all or nearly all true denticles have one or more epithelial rests in close approximation to the border in the same relationship as that of the inner enamel epithelium to the first formed dentine. Calcified nodules in the periodontal membrane may have the same relationship to rests, or the nodule may have the rest as a nucleus into which the lime salts have been precipitated. In the bifurcations of the multirooted teeth, especially of the upper molars, globular deposits of enamel are occasionally found. These are, without exception, associated with larger aggregations of enamel organ epithelium and are usually called enamel pearls. It would seem that their origin is brought about by the activity of that portion of the Hertwig sheath that may have to do with the control of the form and number of the roots. It is possible that at times active ganoblasts are gathered into such a rest, and that in this way the deposition of enamel in a sharply localized spot is brought about. Many of these deposits are so small that they are seen only in histologic preparations.

Odontomas are more likely to develop during the tooth-forming period. They are frequently associated with either missing or retained teeth, and typically they contain all the hard structures of a tooth. Practically the same may be said of dentigerous cysts. Schürmann, Pflüger and Norrenbrock (Die Histogenese ekto-meso-dermaler Mischgeschwülste der Mundhöhle, Leipzig, Georg Thieme, 1931) described two typical odontomas in the hypophysis. These facts support the belief that the epithelial remnants of the Hertwig sheath have nothing to do with either odontomas or dentigerous cysts, both of which are more nearly akin to supernumerary teeth and are to be considered as arising from a developmental fault in a missing temporary or permanent tooth or from an accessory tooth germ. In hypophyseal tumors with supernumerary teeth rather remote from the mouth, the primitive dental lamina, instead of developing and extending into the tooth-bearing zone, becomes caught and retained in other parts of the head which have an embryologic connection with the oral cavity.

The epithelium of the adamantine tumor and the multilocular cyst resembles that of either the enamel-forming portion of the dental follicle or the dental lamina of the very primitive tooth bud. The adamantinoma frequently contains elements that highly resemble the stellate reticulum of the enamel-forming cap. The cells of both tend to a columnar form and are characteristically different from the cuboidal or nearly round or oval cells of the Hertwig sheath or the epithelial débris. On this account it is generally believed that both arise from either the dental lamina or the tooth bud (*Zahnanlage*). There is little reason, therefore, to consider that Malassez' epithelial débris has anything to do with the genesis of either the adamantinoma or the multilocular cyst. The histogenesis of the adamantinoma is still obscure and is worthy of further study.

As to primary carcinoma of the jaw bones, the situation is even less clear. The carcinoma may arise from any epithelium located in this region, including, of course, the epithelial bodies in the periodontal membrane. Such tumors are extremely rare, although they are no less interesting. The presence of epithelium both in normal periodontal structures and in granulomas is almost constant. On this account alone it does not seem reasonable to lay any great stress on the probable connection between such a source of epithelium and carcinoma. Certainly all cases of carcinoma of the jaw bone merit careful study and serious consideration before sweeping conclusions are drawn.

As the epithelium of the granuloma, the odontoma and the mildly malignant adamantinoma arises from parts of the tooth germ, there is a certain resemblance in the pathologic processes in which they are concerned. On the other hand,

there are distinct differences. The epithelium of the granuloma retains most of the characteristic properties of the parent structure; it appears to proliferate only under the irritation of the infection or as a result of increased metabolism; it undergoes cystic degeneration of a wholly benign character, often with the abundant production of cholesterol, and it never produces enamel, although it may stimulate other forms of calcification. The cysts and granulomas which are associated with it are not neoplastic but are inflammatory reactions. In the odontoma the epithelium forms enamel; it proliferates but does not tend to infiltrate. The growth is benign and encapsulated and may undergo cystic change, with the formation of a dentigerous cyst. Adamantine epithelium proliferates and tends to infiltrate, though it may be sharply limited at times. It tends to recur after excision, never produces enamel and is prone to cystic degeneration, with the formation of a multilocular cyst having the same degree of malignancy as the solid adamantinoma. It would seem to be relatively easy to differentiate between the epithelium of the relatively rare adamantinoma and that of the common granuloma.

Malassez' discovery of the epithelial débris in the periodontal membrane is now apparently universally accepted. His theory as to its origin is also in almost equally good standing. On the other hand, his theories as to its significance in pathologic processes have been confirmed only in part and are thought to be limited to certain processes that are inflammatory and have to do with the genesis of the dental granuloma and cyst. The epithelial débris of Malassez and the so-called glands of Serres are distinct entities.

ATYPICAL AMYLOIDOSIS. ELEANOR M. HUMPHREYS.

A man, aged 62, had puzzling symptoms related to the cardiovascular system, the liver and bile passages, and, terminally, the kidneys. These were explained in part by extensive sclerosis of the smaller arteries, associated with thrombosis of a coronary artery, with a myocardial infarct and nephrosclerosis and with marked renal atrophy. The most interesting feature of the autopsy was widespread amyloidosis involving many tissues which are not frequently affected. Amyloidosis of the heart was probably responsible for some of the cardiac symptoms and for the bizarre character of the electrocardiogram. Exceptional features of the case were the unusual amyloid transformation of the testes, the atypical distribution of amyloid in the liver and kidneys and its complete absence from the spleen. Another point of interest was the failure to find a cause for the amyloidosis, unless a seemingly old and healed pleural inflammation or a postoperative subdiaphragmatic abscess which developed two months before death can be implicated. Clinically, there was a marked decrease of the serum protein and of the albumin-globulin ratio, but whether this was related to the amyloidosis is a matter of speculation.

Several similar cases were reviewed.

RETOTHEL SARCOMA. PERRY J. MELNICK.

Retothel sarcoma is a term suggested to Roulet by Rössle. He used it in articles published in 1930 and 1932 to replace the term reticulum cell lymphosarcoma. The derivation is from the German *Retothelien* (reticulum). The term is more accurately used, however, as a contraction of the bulky term reticulo-endothelial, because the reticular element should not be separated from the endothelial element of this system.

Reticulum cell lymphosarcoma, or retothel sarcoma, was recognized as a type of lymphosarcoma by Ghon and Roman in 1916. Because the relationship of the lymphoblast to the reticulum cell was not clear, it had been considered as related to lymphoblastic lymphosarcoma. Roulet clearly demarcated it from the latter. The present study includes four cases which came to autopsy and six biopsy specimens of retothel sarcoma at Cook County Hospital. The diffi-

culty of its histologic diagnosis is emphasized, especially in regard to its differentiation from atypical forms of Hodgkin's disease, from mycosis fungoides and from lympho-epithelioma of the pharynx.

DISCUSSION

VICTOR LEVINE: Retothel sarcomas are not difficult to diagnose. Dr. Melnick will agree that the diagnosis can be made from a simple biopsy.

P. A. DELANEY: I think that terminologists should be commended for their power of invention. I suspected that retothel might be a contraction of the term reticulo-endothelial, but so far as derivation is concerned, it is poor usage. I much prefer the first recommendation of reticulum cell lymphosarcoma.

VARIX OF THE PULMONARY VEIN. BENJAMIN H. NEIMAN.

To the three cases of varices of the pulmonary vein already recorded another is added. This occurred in a white woman, aged 57, who complained of cyanosis, clubbing of the fingers and pain in the chest of several years' duration. Roentgen examination revealed a density at the base of the left lung which, on comparison with a roentgenogram taken three years before, showed no change in size or shape. The diagnosis rested between aneurysm of the descending aorta and benign neoplasm of the lung. Fluoroscopically, it was possible to separate this tumor from the heart shadow and show that it had no relation to the aorta. A huge varix of a branch of the second order of the left upper pulmonary lobe, with mural thrombi, was demonstrated post mortem.

The shadow that such a varix casts on the x-ray film must be differentiated from that seen in primary chondroma and solitary echinococcus cyst of the lung. Although the periphery of all three is nodular, that of the varix is smooth, while the echinococcus cyst usually has slight inflammatory infiltrations in the immediately adjacent pulmonary tissue. The periphery of the chondroma is also smooth, but invariably it shows a patchy, irregular distribution of lime salts, due either to simple calcification of the cartilaginous matrix or to true ossification.

If roentgen examination shows a density with a nodular periphery in the lung, and if there are symptoms of cyanosis, dyspnea, cough, clubbing of the fingers and recurrent hemoptysis, without serologic or clinical evidence of infestation with echinococci, varix of the pulmonary vein should be considered.

Book Reviews

Comptes rendus de la première conférence internationale de pathologié géographique, Genève, 8-10 Octobre, 1931. Publiés au nom du comité directeur de la Société internationale de pathologie geógraphique, par M. Askanazy, Président. Paper. Price, 10 francs (Swiss). Pp. 367. Genève: Librairie Kundig, 1933.

This volume records the results of the first concerted international study of a disease. National committees working in twenty-four countries collected information on the occurrence, clinical manifestations, pathologic anatomy, etiology and other features of cirrhosis. This material has been digested, summarized and compared with observations previously recorded. The results are presented under appropriate subdivisions.

Hepatic changes in cirrhosis are summarized by de Josselin de Jong, Utrecht. Materials from the survey and in the recent literature are correlated. The structural features essential to cirrhosis are: (1) increase in connective tissue, (2) degeneration and destruction of liver cells and (3) regeneration of liver cells. Other changes, such as pigmentation, proliferation of bile ducts, infiltrations and vascular changes, are inconstant though often important features. Cirrhosis develops when, through a long-continued diffuse injury, liver cells are destroyed, followed by regeneration and proliferation of uninjured cells and by infiltration and proliferation of reticulum. The essential character of cirrhosis, a chronic inflammatory process, is obscured by attempts at a rigid morphologic classification. "Il n'y a qu'une cirrhose" (there is only one cirrhosis). Morphologic variations result from variations in the character, the intensity and the duration of the injurious agent and in the physiologic reactions of the individual. The etiology includes those agents which, singly or in combination, may produce chronic diffuse inflammation of the liver. Among these are toxic substances and infections. "The importance of alcohol as an etiologic agent has been greatly overrated."

The grouping of cirrhoses is regarded not as a classification but as a basis for correlating clinical manifestations with structural changes. Laënnec's cirrhosis as a type is not sharply differentiated as to either etiology or clinical or histologic characteristics. No differentiation is made between Laënnec's cirrhosis and fatty cirrhosis. Pigmentary cirrhosis is regarded not as an entity but as a combination of ordinary cirrhosis with metabolic disturbances of uncertain origin. Cirrhosis may produce the clinical syndrome described by Hanot, but his description of the structural changes is not supported by postmortem evidence. No criteria are found by which to differentiate Banti's disease from Laënnec's cirrhosis. The evidence is insufficient for regarding Banti's syndrome as a disease entity. The occurrence of cirrhosis shows striking geographic variations. In Switzerland cirrhosis is found in more than 10 per cent of autopsies; in adjacent countries the occurrence is less than 3 per cent.

Changes in other organs accompanying cirrhosis are summarized by R. Rössle, Berlin. The indefinite etiology of cirrhosis makes it difficult to distinguish between changes genuinely related and those occurring incidentally. Icterus is a feature in from 20 to 50 per cent of cases. Ascites is found in from 20 to 65 per cent of cases. Splenic involvement, consisting of varying degrees of fibrosis of pulp and the occurrence of perisplenitis with adhesions, is frequent. The spleen is increased in size in about 50 per cent of cases. The frequency with which death results from peritonitis, endocarditis or erysipelas is significant.

The clinical phases of cirrhosis are summarized by Noël Fiessinger, Paris. He comments on the geographic and racial variations. Cirrhosis occurs in the white race more frequently than in Negroes, in a ratio of 3:2. Density of population is accompanied by an increased incidence of cirrhosis. Fiessinger believes that alcohol

is an important etiologic factor in Europe, and that in tropical countries parasitic diseases are more important. Syphilis plays an indefinite, minor etiologic rôle. Discussing clinical types, he states that the syndrome of Hanot exists, but without structural alterations of distinctive character. Clinical manifestations and their significance, tests for hepatic function, the relation of splenic disturbances to cirrhosis, the progressive character of cirrhosis and the terminal complications are discussed.

Experimental cirrhosis in relation to the etiology of human cirrhosis is summarized in six pages by W. E. Gye, London. The subject matter is not well chosen. This phase of cirrhosis is inadequately covered.

Disturbances of metabolism in cirrhosis are summarized by F. C. Mann and J. L. Bollman, Rochester, Minn. Material for this report was drawn from experimental work. The cirrhotic process may produce no measurable metabolic effects except as it impairs the secretion of bile or inhibits the regeneration of hepatic cells. A small amount of hepatic tissue may function adequately without evidences of hepatic insufficiency. Acute degenerations of liver cells are usually found. Mann and Bollmann believe that these are not specific for cirrhosis and result chiefly from impairment of biliary secretion. Cirrhosis produces no marked basal metabolic variations. An important feature of cirrhosis is a decreased glycogen content of the liver cells. This diminishes the detoxicating function of the liver and its ability to resist toxic agents and other injuries. Animals with greatly reduced hepatic tissue do not survive on a diet high in protein. They react severely to doses of toxic substances which are harmless to normal animals. They succumb to surgical procedures from which normal animals recover. The liver has numerous metabolic functions. All of these will be affected by a serious reduction of hepatic tissue.

A report on the frequency and forms of cirrhosis in Stockholm by F. Henschen and T. Bruce and one on statistical data, classification and etiology by P. Robert, Zurich, complete the survey. A verbatim report is made of all discussions. The conference was trilingual: French, German and English. Each item is recorded in the language used in its presentation. The reports reflect a transition of opinion as to etiology, forms and interpretations. Those interested in the morphologic, physiologic or clinical features of cirrhosis will find valuable material in this book.

The organization of the Association was completed and its administration placed in the hands of a directoral committee in which France, Germany, Holland, Switzerland and the United States are represented. An international conference will be held triennially. The second conference will meet in July, 1934, at Utrecht, de Josselin de Jong, president, presiding; the subject is arteriosclerosis. It is gratifying to note that there is an international association for the coordinated study of the various phases of pathology. Its method of procedure promises programs of unique character and high quality.

Die normale und pathologische Physiologie der Milz. By Dozent Dr. Ernst Lauda, Assistent der il. medizinischen Universitätsklinik in Wien. Price, 18 marks. Pp. 277, with 2 illustrations. Berlin: Urban & Schwarzenberg, 1933.

This book presents a good review of the literature on the functions of the spleen in health and in disease. The scope is indicated by the list of topics considered in separate chapters: the spleen as a reservoir for blood, particularly red cells; the formation of blood in the spleen; splenic hemolysis; the protective functions of the spleen against infections; the resistance of the spleen to the growth in it of malignant tumors; the relations of the spleen and the gastro-intestinal tract; the spleen and metabolism; the internal secretion of the spleen. At the end of each chapter are a succinct summary of the contents and a useful bibliography. The final chapter gives a cautiously worded digest of the review.

Lauda's book has solid worth for those who are interested in the spleen. The functional relations of this structure are complex; it shares important functions with the reticulo-endothelial system and perhaps also with other systems, and acts too as a more or less independent functional unit. There is, however, no disease so far as is known that is due to hypofunction or hyperfunction of the spleen. Lauda finds that the claims for hyperfunction in certain hemolytic states and in thrombopenia have not been established. Possibly polycythemia as seen occasionally after splenectomy and in splenic tuberculosis may be due to the loss of splenic function. In the embryo the spleen is one of the seats of formation of red cells and myelocytes, and in postembryonal life it shares in comparatively moderate degree in the production of lymphocytes. In infection the spleen has preventive functions of varying significance; it is an important factor in the elaboration of antibodies; it is reasonable to assume that the great splenic swelling in certain infections is associated with increase in protective function; in infection with Bartonella in rats the protective action of the spleen is life-saving, and this appears to be the spleen-specific function that is best established. The spleen is of importance as a depot or reservoir for red cells, notably in the dog. Splenectomized rats succumb to poisoning with carbon monoxide, whereas the normal rat survives. The spleen also appears as an independent unit in its relations to malignant tumors and freedom from metastasis. The appearance of Howell-Jolly bodies in the circulating blood after splenectomy is mentioned as perhaps also an indication of a special splenic function. The book is an important and helpful addition to the literature on the spleen.

Bacterial Infection. With Special Reference to Dental Practice. By J. L. T. Appleton, Jr., B.S., D.D.S., Professor of Microbiology and Bacteriopathology, the Thomas W. Evans Museum and Dental Institute, School of Dentistry, University of Pennsylvania. Second edition, enlarged and revised. Cloth. Price, $7. Pp. 654, with 122 engravings and 4 colored plates. Philadelphia: Lea & Febiger, 1933.

The purpose of this book is "to aid the reader to form a comprehensive concept of infection" and "to point out wherever a knowledge of infection will help the dentist in understanding or solving his problems." The author takes it as granted that if there is to be close and effective cooperation between dentist and physician, the dentist must have a knowledge "equivalent to that possessed by the physician, of what infection means." The book is in three parts. The first part deals with the morphology, physiology and ecology of bacteria. The second part treats of infection as a whole, its nature, modes of action, types, dissemination; the protection and responses of the host; immunity; prevention, including oral hygiene. The third part is devoted to a comprehensive presentation of the infections of the oral cavity. Most of the illustrations are taken from other books. There are abundant references to the literature. The style is clear, fluent, readable. The author shows a remarkable familiarity with the current literature, especially the English and German. The use of italics in cumbersome scientific or proper names of common bacteria, when not required for purposes of classification or identification, is not desirable. Why say *Corynebacterium diphtheriae, Eberthella typhi, Escherichia coli, Myobacterium tuberculosis* and *Neisseria gonorrhoeae* when diphtheria bacillus, typhoid bacillus, colon bacillus, tubercle bacillus and gonococcus will answer the purpose fully? Generally speaking, the teachings of the book are sound. It contains an enormous amount of detailed, minute information about infection and allied topics. As a textbook for students it is too elaborate. To require students of dentistry to master its contents is neither humane nor good pedagogics. As a book of reference for dentists and physicians it merits recommendation. In future editions the effort should center on condensation, simplification and sound generalizations.

The Great Doctors. A Biographical History of Medicine. By Dr. Henry E. Sigerist, Professor of the History of Medicine, The Johns Hopkins University. Translated by Eden and Cedar Paul. Price, $4. Pp. 436, with 68 illustrations. New York: W. W. Norton & Company, Inc., 1933.

This book is a translation from the second German edition. The first edition was published in 1931 and was well received. As indicated in the subtitle, a biographic history of medicine is given, made up of short sketches, never longer than ten or eleven pages, of fifty great figures of vital importance in the development of medicine. The first chapter presents Imhotep and Aesculapius, the second Hippocrates and the late Osler. The plan of the book excludes more than passing allusion to names of living persons; there is no aim at encyclopedic completeness; attention has been restricted to a small number of creative masters of representive significance in each epoch. It does not seem advisable to consider each of these sketches in detail. They are written by a ready and competent pen. The sketches of the following are mentioned as of special interest to pathologists in the strict sense: Morgagni, Rokitansky, Bernard, Virchow, Pasteur and Koch. By giving the subject of each sketch the proper relative historical setting, a broad conception is conveyed of the growth of medical knowledge. The book is dedicated "To the Unknown Doctor who in unselfish and inconspicuous activities fulfills the teachings of the Great Doctors." The illustrations, sixty-eight in all, add greatly to the interest of the book. Physicians and medical students will find this a book of exceptional interest and stimulus.

Books Received

THE ERADICATION OF BOVINE TUBERCULOSIS. L. Jordan. Medical Research Council, Special Report Series, No. 184. Price, 2s., net. Pp. 104. London: His Majesty's Stationery Office, 1933.

METABOLIC DISEASES AND THEIR TREATMENT. Dr. Erich Grafe, Professor of Medicine of the Clinic of Medicine and Neurology, University of Würzburg, Germany. Translated by Margaret Galt Boise under the supervision of Eugene F. DuBois, M.D., Medical Director, Russell Sage Institute of Pathology; Professor of Medicine, Cornell University Medical College, New York, and Henry B. Richardson, M.D., Associate Professor of Medicine, Cornell University Medical College, New York. Price, cloth, $6.50, net. Pp. 551, with 37 illustrations. Philadelphia: Lea & Febiger, 1933.

HISTOLOGY. S. Ramón y Cajal, M.D. (Madrid), F.R.S. (London), LL.D. (Clarke), Director, Royal Cajal Institute for Medical Research; Emeritus Professor of Pathology, University of Madrid Faculty of Medicine; Nobel Premiate in Medicine; Life Senator of Spain. Revised by J. F. Tello-Munoz, M.D. (Madrid), Professor of Pathology, University of Madrid Faculty of Medicine. Authorized translation from the tenth Spanish edition by M. Fernán-Nunez, M.D. (Madrid), Professor of Pathology, Marquette University Medical School. Price, $8. Pp. 738, with 535 illustrations. Baltimore: William Wood & Company, 1933.

MEDICAL USES OF RADIUM: SUMMARY OF REPORTS FROM RESEARCH CENTRES FOR 1932. Medical Research Council, Special Report Series, No. 186. Price, 1s., net. Pp. 36. London: His Majesty's Stationery Office, 1933.

DIET AND HEALTH. Milton T. Hanke. Pp. 236, with illustrations. Chicago: University of Chicago Press, 1933.

STUDIES FROM THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF PENNSYLVANIA. Volume IV. Edited by E. B. Krumbhaar, Professor of Pathology. Philadelphia: University of Pennsylvania, 1932-1933.

THE CHEMISTRY OF FLESH FOODS AND THEIR LOSSES ON COOKING. R. A. McCance and H. L. Shipp. Medical Research Council, Special Report Series, No. 187. Price, 2s. 6d., net. Pp. 146. London: His Majesty's Stationery Office, 1933.

NUMBER 4 CANADIAN HOSPITAL: THE LETTERS OF PROFESSOR J. J. MACKENZIE FROM THE SALONIKA FRONT. With a memoir by his wife, Kathleen Cuffe Mackenzie. Price, $2.50. Pp. 247, with 13 illustrations. Toronto: Macmillan Company of Canada, 1933.